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CORONARY BLOOD FLOW AND THE PATHOGENESIS OF CATECHOLAMINE CARDIOMYOPATHY

Michael Simons

1984

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CORONARY BLOOD FLOW
AND THE
PATHOGENESIS OF CATECHOLAMINE CARDIOMYOPATHY

A Thesis Submitted to the Yale University
School of Medicine in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Medicine.

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Michael Simons

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ABSTRACT

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Norepinephrine (NE) causes extensive myocardial injury in the rabbit. To investigate a potential mechanism, coronary blood flow (CBF) was measured with radioactive microspheres. Coronary resistance (CR) and oxygen demand (HR-BP double product) were calculated. Severity of injury was determined by semiquantative histological evaluation. Changes resulting from NE infusion were determined in animals with or without alpha receptor blockade with phentolamine (Ph, 10mg). In all animals oxygen demand remained constant. CBF (ml/g/min), was 2.66 at the beginning of the experiment, rose to 3.46 after 3 minutes of NE infusion ($p < 0.05$), then declined to baseline values, (2.33) after 10 minutes. However, CBF was sharply lower than baseline (1.51; $p < 0.05$) after 40 minutes of infusion. CR rose progressively from baseline values of 40.9 (units) to 74.8 at 40 minutes ($p < 0.05$). Animals given Ph manifested none of these changes. Saline-infused controls showed no significant

changes in CBF or CR. Animals pretreated with Ph had minimal histological damage at 48 hours compared with NE alone ($p < 0.01$). It is concluded that NE induces sustained coronary vasoconstriction in the rabbit and that reduced CBF may contribute to the pathogenesis of NE cardiomyopathy in this species.

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INTRODUCTION

This thesis deals with the subject of regulation by adenosine of transmyocardial coronary flow and the role of alterations of such regulation in the pathogenesis of catecholamine cardiomyopathy.

Chapter One reviews the literature on local control of coronary flow with a special emphasis on adenosine's role in transmural flow distribution. Chapter Two considers the subject of catecholamine cardiomyopathy. The pathology and pathophysiology of catecholamine injury are reviewed and the possible pathogenic mechanisms are discussed. Chapter Three reports the experimental study designed to determine if the catecholamine-mediated changes in the coronary circulation play a role in pathogenesis of this cardiomyopathy.

It has been established that under normal conditions, as well as under conditions of physiological stress (i.e. exercise), coronary flow is distributed equally between the subendocardial and the subepicardial regions. Since most of coronary flow to the endocardium occurs in diastole, some adaptive mechanisms must operate in order to assure the adequacy and equality of the endocardial perfusion. One such mechanism is a relatively higher vascular density in the subendocardium resulting in lower subendocardial resistance to flow. While this anatomic difference may suffice to supply enough blood to the subendocardium during resting conditions, it is not adequate during stresses such

as exercise or tachycardia. Therefore, one must postulate the existence of an active vasomotive agent, capable of precise adjustment of the transmyocardial flow distribution. Among the many substances proposed for this role adenosine appears to be the most likely candidate.

In Chapters Two and Three we examine the potential role of catecholamine-induced coronary flow changes in the pathogenesis of catecholamine cardiomyopathy. This form of myocardial injury has been well studied morphologically and functionally, but its pathogenesis still remains controversial. One of the characteristic pathological findings in this condition is the predominance of damage in the subendocardial areas. Although morphologically the injury does not present an ischemic pattern any agent inducing damage consequent to a reduction of coronary flow would be expected to produce the most pronounced injury in the subendocardial regions. Recently it was found that alpha-adrenoceptor activation mediates the damage and that alpha-blockade effectively prevents its development. Thus it can be speculated that norepinephrine induced coronary vasoconstriction is responsible for pathogenesis of the lesions.

A number of studies have addressed the question of norepinephrine induced coronary flow changes, and the most frequent finding is that the coronary flow actually increases. It should be noted, however, that these studies

were largely concerned with acute effects of norepinephrine and no long term infusions were performed. It is, therefore, possible that a longer term exposure to norepinephrine might actually decrease coronary flow. The reason why a short term infusion would result in increased flow while a long term one reduces the flow may lie with the interaction of norepinephrine with adenosine.

Adenosine is known to counteract many of the myocardial effects of catecholamines. Furthermore, norepinephrine administration has been shown to cause adenosine release by myocardium. Thus, norepinephrine induced adenosine release could potentially override the vasoconstrictor effects of the amine and result in increased coronary flow. This would explain the finding in studies dealing with short term norepinephrine infusion. However, long term infusion of norepinephrine results in a significant reduction of coronary blood flow, as was found in the present study. This may in part be due to loss of the protective effect of adenosine.

CHAPTER ONE

LOCAL CONTROL OF TRANSMYOCARDIAL CORONARY BLOOD FLOW

1 Funcional Anatomy of the Coronary Circulation

The major coronary vessels pass on the epicardial surface of the heart from the AV ring to the apex. They divide on the epicardial surface, and after reaching the diameter of 3 mm, begin to give off branches at right angles into the myocardium. These penetrating branches have the diameter of 400 to 1500 micron and fall into two general classes: class A and class B (1, 2). Class A vessels quickly branch into a very fine network which is distributed in the outer 3/4 to 4/5 of the myocardium. Class B vessels are less numerous, branch infrequently and reach the inner layers of the myocardium with no reduction in size. In the subendocardial layer these vessels subdivide forming a large meshwork of anastomoses. Therefore, this large endocardial plexus is supplied at multiple points by class B vessels derived directly from epicardial vessels.

Class B vessels of the subendocardial plexus typically range in size from 50 to 500 microns, with occasional vessels in the plexus reaching the diameter of 1000 microns (1). The capillary network thus formed in the myocardium has the average density of 4000 capillaries/mm (3). Capillaries run parallel to the myocardial fibers, and, as viewed in cross-section, there is about one capillary for

each fiber. This places each fiber in contact with 3-4 capillaries (3). Under normal physical conditions not all capillaries are open, and additional ones can be recruited depending on the myocardial needs (4,5).

The normal heart possesses some connections between distal branches of major coronary arteries. However, these anastomoses are insufficient to maintain adequate perfusion following a sudden occlusion of a major artery (6).

An important question arises concerning the ability of these anastomoses to maintain some degree of perfusion for a certain period of time after an occlusion of a major coronary artery. This question has important clinical implications, for if such a region at risk ("border zone") existed some kind of intervention designed to salvage myocardium in this area could be attempted, while if such a zone did not exist, nothing short of immediate reperfusion would suffice. A considerable controversy has arisen over the existence of such a border zone of intermediate injury during regional myocardial ischemia. Although a detailed review of this subject is beyond the scope of this chapter, the subject illustrates well some important aspects of methodology as well as of functional anatomy of coronary circulation.

2 Border Zone

Basically, two possible situations exist. If a major coronary artery is suddenly occluded, a zone of ischemia

results. Now, going from this zone of ischemia in the lateral and transmural planes, one may observe either a sharp transition to the normal tissue or, alternatively, a zone with intermediate levels of flow, metabolism and injury- a border zone. This intermediate zone is thought to exist because of collateral circulation from the nonoccluded artery, which is sufficient to maintain its viability at some intermediate level for a certain period of time. It should be pointed right away, that border zone is a spatial as well as a temporal category. The existence of a spatial border zone would be determined by a variety of anatomic factors, while the existence of a temporal border zone is dependent on a variety of biochemical changes and the dynamic nature of the ischemic process.

2.1 Spatial considerations

A large number of studies attempted to demonstrate the existense of a border zone lateral to the area of ischemia. This was attempted by measuring coronary blood flow, levels of ATP, lactate, NAD, as well as PO2 and ST segment changes.

Barlow and Chance (7) used surface fluorescence technique to visualize lateral and transmural gradients of NAD reduction. On the basis of the obtained data they postulated the existence of a significant border zone.

Hearse et al. (8) used a specially designed biopsy instrument with multiple drill heads to obtain simultaneous

multiple transmural biopsy samples. They identified a 8 to 15 mm boder zone characterized by intermediate levels of coronary flow, ATP, creatinine phosphate and lactate concentrations.

Vokonas et al. (9) used 14C- antipyrine radioautography to assess regional flow after an acute coronary artery occlusion. Twenty micron wide sections were made in transverse and transmural planes. Using these methods the authors found a 4.5 mm wide border zone of intermediate flow.

Jugdutt et al. (10) used planimetry from "bread loaf" sections to calculate the size of infarct area and of the border zone wich was found to be of significant size. All these studies, however, suffered from a common methodological problem, namely that measured intermediate levels of metabolites and coronary flow could result not only from the existence of cells with truly intermediate metabolic states, but also from the coexistence of critically injuried cells along- side healthy and viable ones.

It is apparent that a spatial reconstruction of the infarct area and the surroundings is necessary to distinguish these two possibilities. Factor et al. (11) performed serial sections of dog heart 24 hours after coronary occlusion reconstructing three dimensional geomety of the infarct area using staining techniques. They found

that areas of normal tissue in the ischemic zone that used to be considered the remaining islands of viable tissue supplied by the occluded artery are in fact complex interdigating peninsulas of normal tissue supplied by another coronary artery invading the ischemic zone.

Hirzel et al. (12) found no lateral border zone of intermediate creatinine kinase depletion 24 hours after coronary occlusion in dogs when tissue supplied by non-occluded artery was excluded.

Okun et al. (13) and Factor et al.(14) using colored Microfil injections in canine and human hearts respectively, found that capillaries derived from one coronary artery form descrete loops without interconnection or anastomoses with capillaries derived from another coronry artery. Using the same technique, Factor et al. (15) found a close correlation between the distribution of intramyocardial vasculature and the histological myocardial infarction at the infarct margin 24 hours post occlusion.

Thus, a firm morphological basis for a sharp lateral infarct margin has been established. It has still been necessary to demonstrate that functionally this margin was also sharp. Yellon et al. (16) used multiple continuous biopsy device to assess metabolic changes in transmyocardial biopsies. They found no border zone of intermediate reduction of metabolism. Murdoch et al. (17) used post mortem angiograms to separate regions perfused by occluded

and nonoccluded coronary arteries. Three millimiter wide sections were obtained. It was found that an itermediate reduction in flow occured in a very narrow rim of myocardium less than 3 mm wide, immediately inside the ischemic zone, and represented an admixture of normal myocardium to the ischemic area.

2.2 Temporal considerations

It can be considered established that the lateral border of an ischemic area is sharp and the observed intermediate levels of metabolism and flow are due to the presence of peninsulas of viable tissue among the dead cells. However, the existence of a transmural border zone is a somewhat different matter. Two possible scenarios can be envisioned. One possibility is that upon occlusion of a coronary artery an entire thickness of the myocardium becomes an area at risk and with a passage of time the entire area will necrose. Thus, in this model, border zone is exclusively time- dependent. Alternatively, a spatially distinct zone of cells at risk may exist within the ischemic region. The border of this area will, however, move with time (18, 19). It is not clear which of these two possibilities is actually correct and the question of transmural border zone will require further studies.

3 Coronary Blood Flow

The factors determining the overall magnitude of coronary blood flow are the perfusion pressure and coronary resistance to flow. By analogy with Ohm's Law, coronary blood flow is directly proportional to the perfusion pressure and inversely proportional to the resistance to flow ($Q=P/R$). We will discuss the factors determining the perfusion pressure and flow resistance separately.

3.1 Perfusion Pressure

The perfusion pressure responsible for coronary blood flow is the pressure gradient between the ostia of the coronary arteries and the coronary sinus. The pressure at the coronary ostia varies somewhat with the cardiac cycle. In systole, ostia are partially occluded by the aortic valve leaflets. The effective pressure in the ostia is slightly less than the systolic aortic pressure because of Venturri effect (20). In diastole aortic pressure (diastolic) is effectively transmitted unchanged to the ostia. Obviously, any obstruction at the ostia will reduce the perfusion pressure.

The coronary sinus pressure also varies somewhat with the heart cycle. Increase in the right atrial pressure leads to a decrease in coronary perfusion pressure. It has been recently shown that an increase in coronary sinus pressure raises the diastolic pressure-zero flow intercept in the

subepicardium, while in the subendocardium the pressure-zero flow intercept is not changed. This increases the endo/epi flow ratio (21). These findings appear to support the waterfall model of coronary circulation (see below). Interestingly, increase in coronary blood flow due to increased perfusion pressure was also shown to result in the increased endo/epi flow ratio (22).

3.2 Resistance to Flow

The resistance to coronary blood flow can be viewed as a sum of two components: resistance due to factors extrinsic to the coronary bed and resistance due to factors intrinsic to the coronary bed. Factors extrinsic to the coronary bed include mechanical deformation of the coronary arteries by the myocardium while factors intrinsic to the coronary bed include influences affecting the contractile state of smooth muscle in the walls of coronary arteries. Each of these two resistance components are discussed below.

Factors Extrinsic to the Coronary Vascular Bed

The major factor responsible for extrinsic resistance to coronary blood flow is myocardial tension in systole. The fact that systole affects coronary flow can be dramatically illustrated by inducing asystole and observing sudden surge in coronary flow (23, 24). Accordingly, a large fraction of coronary flow to the left ventricle occurs in diastole. Compressive forces in the right ventricle affect the

systolic coronary flow to a much leser extent than in the left ventricle (25).

It has been estimated that under resting conditions the extrinsic resistance accounts for 25 per cent of the total coronary flow resistance (26). Since systole occupies a larger portion of the cardiac cycle, it follows that some coronary blood flow does take place during systole. Any factor that increases the proportion of the cardiac cycle occupied by systole relative to diastole will, therefore, tend to compromise coronary blood flow.

Tachicardia, for example, reduces coronary blood flow through this mechanism. (Tachicardia also increases a myocardial oxygen demand while compromising oxygen delivery). Other conditions prolonging systole include increased afterload (aortic stenosis, hypertention, etc.), decreased contractility, dilatation or hypertrophy.

Systolic extracoronary resistance.

Systole can impede coronary flow by two mechanisms: 1) development of intramyocardial shearing forces that could kink vessels traversing the myocardium and 2) transmission of intramyocardial tissue pressure to the vessels.

As was described in the section dealing with coronary vessel anatomy, arteries supplying the endocardial plexus take off from epicardial vessels at right angles and go straight through the myocardial wall with almost no

branching. On the other hand, vessels supplying the outer one half to two thirds of the myocardial wall branch extensively in the epicardial layers after the take off from large coronary vessels. Therefore, any deformation of the intramyocardial vessels in the midwall will have its greatest impact on the subendocardium.

The LV wall is made of multiple muscle fibers whose orientation changes in a rotational manner with the myocardial depth (27). It is known that this rotational change causes shear strains between the adjacent layers. Therefore, it is conceivable that these strains could kink or twist arteries passing through the tissue. Downey et al. (28) examined effects of shear strains on the systolic extravascular resistance. They found that shearing forces resulting from systolic shortening accounted for only a small fraction of the total resistance, and that effects of these shearing forces were distributed uniformly through the LV wall, and, therefore, could not account for the systolic endo/epi flow gradient. An additional argument against the major role of shearing strains comes from studies of Hess and Bache on right ventricular systolic flow (25). They showed that no transmural flow gradient developed in the right ventricular wall when coronary flow was confined to systole. Recently, Williams et al (29) examined the transmyocardial coronary flow distribution in the empty beating heart using low perfusion pressure and maximal vasodilation. Such a preparation maximizes systolic strains

in the myocardial wall by allowing the heart to contract against a zero afterload. They found that a small transmural flow gradient which was nowhere near in magnitude the gradient developing during systole in normally beating hearts contracting against a ventricular pressure. Therefore, it is unlikely that systolic shearing strains play a significant role in the development of extravascular coronary resistance.

We are left, then, with intramyocardial systolic tissue pressure as the major factor accounting for the systolic extravascular resistance. It has been conclusively shown that when left ventricular coronary flow is restricted to systole, a substantial transmural flow gradient develops with epicardial flow far exceeding the endocardial flow (30-32). Therefore, it is logical to assume the existence of a systolic intramyocardial pressure gradient between the endocardium and the subepicardium, with subendocardial pressures exceeding subepicardial pressures. The magnitude of this gradient, however, remanis a subject of considerable disagreement.

Kirk and Honig used the fluid-filled pocket model in 1964 (33) to find that subendocardial pressure in systole exceeded intracavitary systolic pressure. At the same time they observed that subepicardial systolic pressure was less than intracavitary (and subendocardial) systolic ppressures. Brandi and McGregor suggested in 1969 (34) that the

introduction of even a small volulme distorts intramyocardial tissue architecture, and, therefore, different tissue pressure will be obtained with fluid pockets of different sizes. By using different rates of infusion, creating thereby pockets of varying volumes, and measuring intamyocardial pressures at these different pocket volumes, they came to the conclusion that extrapolating to intramyocardial pressure at zero pocket volume results in subendocardial pressure equal to intracavitary pressure in systole. They have also found that systolic subepicardial pressure was equal to the ambient pressure. Armour and Randall used miniature pressure transducers in 1971 (35), while Stein et al. used micropressure transducers in 1980 (36) to obtain systolic subendocardial pressures in excess of intracavitary systolic pressure. Although it can still be argued that microtransducers distort tissue architecture and, therefore, are not very accurate, there is no doubt that substantial transmural pressure gradient exists in the LV wall with subendocardial pressures likely exceeding intracavitary pressure and subepicardial pressures less than intracavitray and subendocardial pressures. On the other hand, a waterfall theory proposed by Downey and Kirk (discussed in detail below) predicts that the magnitude of the subendocardial pressure should be very close to the interventricular pressure. This prediction was tested recently in a study by Munch and Downey (37), who measured blood flow to maximally dilated subendocardial vessels under

a variety of hemodynamic conditions. They found that a waterfall model predicted the value of the subendocardial flow accurately when the subendocardial pressure was taken to be equal to the intracavitary pressure. On the other hand, when the subendocardial pressure was taken to be twice the magnnitude of the intracavitary pressure the correlation between the observed and predicted subendocardial flow was poor (37).

Diastolic extracoronary resistance The subject of diastolic extravascular resistance is less well studied and understood than that of systolic resistance. The diastolic intramyocardial pressure can be thought of as consisting of two components: intrinsic pressure (intramyocardial pressure at zero preload) and the pressure transmitted into the myocardium from the diastolic chamber pressure (preload component) (38). There exists a considerable controversy regarding the magnitude of diastolic intramyocardial pressure, its transmural distribution and its importance in the overall diastolic blood flow regulation.

While some investigators found that diastolic intramyocardial pressure is uniformly distributed through the ventricular wall (39), others found a transmyocardial pressure gradient with subendocardial pressures exceeding subepicardial ones (40,41). Stein et al. (36) used implantable pressure microtranducers to find that the diastolic subepicardial pressure was higher than

subendocardial pressure, which in turn was higher than the end-diastolic intracavitary pressure. Therefore, they in fact observed a diastolic transmural pressure gradient across the myocardial wall, which was opposite in direction to the systolic transmural pressure gradient. It is important to note that while Stein et al. and Baird et al. (36, 39) used direct pressiure measurements, Pao et al. and Rouleau et al. (40,41) used such indirect methods as pressure-zero flow intercept to estimate the diastolic intramyocardial pressure. The difference between direct and indirect studies will have to be resolved.

The relative importance of diastolic extravascular resistance versus intravascular resistance is a matter of another controversy. Although it is generally assumed that the diastolic resistance to coronary blood flow is primarilly determined by the coronary vessel tone (intravascular resistance), Archie (38) found that diastolic extravascular resistance was a major determinant of diastolic coronary flow.

Factors Intrinsic to the Coronary Vascular Bed

Neural, metabolic, pharmacologic and myogenic factors influence the tone of myocardial vessels (42,43). There is evidence both for and against tonic coronary vasoconstriction mediated by sympathetic nerves (44). Stimulation of parasympathetic centers leads to reflex vasodilation (44a). Baroreceptor activity can also affect

coronary flow by reducing coronary vascular resistance. Adrenergic stimulation and blocade can also modify coronary vascular tone. However, these factors are not likely to play a major role in the beat-to-beat regulation of coronary blood flow and their contribution even under pathological conditions is probably a modest one. Several recent reviews (42,43,45). deal extensively with this subject.

4 Autoregulation of Coronary Blood Flow

Sudden alterations in coronary flow induced by extracardiac stimuli are transitory, and the flow quickly returns to the baseline level. This mechanism, termed autoregulation, tends to maintain coronary flow within a relatively narrow range, regardless of changes in the perfusion pressures. In fact, it can be demonstrated that the CBF remains relatively constant over a wide range of blood pressure changes (41,20). Two mechanisms were proposed to explain how autoregulation works- myogenic and metabolic. Each will be discussed in turn.

4.1 Myogenic Factors

It is known that transitory stretch of vascular smooth muscle resulting from an increase in perfusion pressure stimulates the muscle to contract. This leads to an increase in the vascular resistance which in turn leads to a decrease in the blood flow through the vessel. Decreased perfusion pressure, on the other hand, leads to a vasodilation secondary to a reduced smooth muscle stretch.

In coronary vasculature, reactive hyperemia results in blood flow far in excess of the flow deficit incurred during the period of occlusion, typically 400 - 600 per cent more than the accumulated blood flow debt (46). A close examination of this phenomenon revealed that the coronary reactive hyperemia includes separate flow and time

components. The flow component is due to myogenic vasodilation and is approximately equal to the flow deficit incurred. The time component is due to the time interval required for a washout of different vasodilator metabolites (46). Although the myogenic mechanism may contribute to the vasodilator response, its role in coronary flow regulation is probably a modest one (20).

4.2 Metabolic Factors

A number of mediators are effective in the regulation of CBF, including lactate, carbon dioxide, PO2, K+, H+, prostoglandins and adenosine.

Although hypercapnia can induce vasodilation (47), it is unlikely that it plays a major role in the CBF regulation, because its vasodilator potential is quite low (48).

Potassium ions are known to be able to cause coronary vasodilation (49). However, potassium release in the abscence of ischemia is usually transient and thus it cannot account for continuing CBF regulation under normal conditions (49).

Although it was proposed that p02 is directly responsible for the control of coronary vascular tone (50), the bulk of the available evidence suggests that p02 per se plays little or no role in coronary flow regulation (42).

As could have been expected, prostaglandins were proposed as mediators of the CBF regulation. Current evidence suggests, however, that prostaglandins play no major role in coronary flow regulation (43).

4.3 Adenosine

Adenosine's role as a major regulator of coronary flow was proposed and vigorously defended by Berne and associates (51-57). The adenosine hypothesis states that adenosine, produced from intracellular adenine nucleotides is continuously released from myocardial cells into interstitial fluid, and tha increased MVO2 or reduced oxygen supply leads to an increased adenosine release (57).

In order to play this role adenosine must meet the following criteria (57):

1). It must be a potent coronary vasodilator.

2). It must have an endogenous source.

3). It should be present under normal physiological conditions.

4). Its concentration in the interstitial fluid should be sufficient to induce vasodilation.

5). There should be a close relationship between adenosine's interstitial fluid concentration and coronary flow.

6). The time course of adenosine's action should parallel the changes in coronary flow.

7). Agents that potentiate or attenuate the action of adenosine should have a similar effect on endogenously released adenosine.

8). A direct cause and effect relationship should be established.

Although adenosine appears to fullfil most of these criteria, almost all aspects of its physiology remain a subject of considerable controversy.

Originally adenosine was proposed as a mediator of ischemia-induced vasodilation (51,52). However, later its proposed role was extended to include the mediation of coronary blood flow in response to increased cardiac performance and other non-ischemic stimuli. This aspect of adenosine's function is the subject of the most controversy. In a large part it is due to the differences in techniques used in estimation of adenosine concentration. It is, therefore, important to consider in detail the different approaches and methods used for this purpose.

Since it is assumed that adenosine in the interstitial flud (ISF) is responsible for arterial vasodilation, it becomes important to measure as accurately as possible ISF adenosine concentration. Because there are no ways of direct sampling of the ISF adenosine, indirect estimates must be used. There are three way to do that: direct tissue sampling, coronary sinus-arterial blood adenosine concentration difference, and pericardial infusate technique.

Direct tissue sampling was probably the first method used for estimation of adenosine concentration. This method

is based on the assumption that virtually all existing adenosine is produced in the extracellular space by the action of an ecto enzyme, membrane-bound 5'-nucleotidase (5'-NT). Recent data, however, show that this, perhaps, is not the case. First, extrapolation of ISF adenosine concentration from the total tissue adenosine content grossly overestimates it, for there would be enough adenosine even under basal conditions to produce maximal vasodilation (58,59). Furthermore, analysis of breakdown products of prelabeled adenine nucleotides (58) and of adenosine content in the pericardial fluid (59) suggest that adenosine to a substantial extent may be localized in the intracellualr compartment. Thus, Scharader and Gerlach using prelabeled adenine nucleotides found that at least two different adenine nucleotide compartments of the heart serve as precursors for the formation of adenosine, one characterized by high and the other by low specific ativity. The fraction with high specific activity appeared to be responsible for adenosine production under normal conditions, while during ischemia a fraction with low specific activity appeared to contribute substantially to the total adenosine pool. Both of these fractions appeared to be localized intracellularly (58). Olsson et al (59) found that treating enzymatically dispersed myocardial cells with adenosine deaminase destroyed > 99% of exogenous adenosine, but the total tissue adenosine content still remained high. This was interpreted by authors to indicate

the existence of a large intracellular adenosine pool. Schutz et al. (60) studying isolated guinea pig hearts found that an intracoronary infusion of a potent 5'-NT inhibitor, alpha-beta-methylene adenosine 5-diposphate (AOPCP), which inhibited the activity of myocardial ecto-5'-NT by 85%, did not influence the release of adenosine provoked by accelerated heart rate during hypoxic perfusion. On the other hand, intracoronary infusion of nucleoside transport inhibitor 4-nitrobenzyl thionosine (NBMPR) resulted in decrease of hypoxia-induced adenosine release.

The presented evidence for intracellular formation of adenosine seems rather compelling. However, cytosol is known to contain high concentration of adenosine deaminase which would immediately degrade any adenosine produced intracellularly. Therefore, one must postulate that intracellular adenosine is bound to proteins or some other buffer, that prevents its degradation. Whether adenosine stored in such a form is readily available for release into the extracellular space (a step necessary for coronary flow regulation) is completely unknown. Thus, it appears that the total tissue adenosine concentration exaggerates ISF adenosine content and, at present, cannot be used as a relia- ble indicator of it. The subject of intracellular adenosine storage and its release clearly requires further study.

The other way of estimation of ISF adenosine

concentration involves calculation of coronary sinus-arterial blood adenosine concentration difference. As could have been expected, there are problems with this method as well. Adenosine may be enzymatically degradable in blood, it can be taken up by erythrocytes and endothelia cells, and the rapidity of its exchange during passage through a capillary bed may be insufficient (61). Rubio et al. (62) measured the metabolism of adenosine added to the dogs blood. They found that 65% was metabolized in 10 seconds. In this study, the adenosine metabolism at the end of 10 seconds was stopped by diluting the blood with ice-cold saline, which could have been inadequate for eliminating of all enzymatic activity. Olsson et al (63) deproteinized blood samples 10 seconds after the addition of adenosine. They found that 15-21% of added adenosine was degraded in 10 seconds. Manfredi and Sparks (64) added dipyridamole, ethanol, and EHNA, an inhibitor of adenasine deaminase to stop adenosine metabolism in the whole blood. They found that about 16% of adenosine is degraded in 10 seconds. Klabunde and Althouse (65) using methods similar to Olsson et al., found that the mean adenosine half-time was 3.42 min. with only 3% being metabolized in the first 10 seconds. However, adenosine half-life in different dogs in the study varied from 1.1 to 7.19 min. Such a wide variation devoids the mean value of any physiological significance, and indicates that each dog should be assessed separately.

Thus it remains uncertain whether the coronary sinus - arterial blood adenosine concentration difference accurately reflects the ISF adenosine concentration, because of possibly substantial adenosine metabolism by the whole blood and wide differences in this enzymatic ability between different animals. Anyone attempting to estimate ISF adenosine concentration using this method should probably estimate each dog's enzymatic capability for adenosine degradation individually.

The last method used for estimation of ISF adenosine concentration is a pericardial infusate technique developed by Berne's group. The method involves infusing 40 cc of modified Krebs-Henseleit solution which is withdrawn 4.5 minutes later. Adenosine concentration is then determined in this infusate. This technique has its own share of problems as well. First, adenosine in the pericardial infusate arises exclusively from the epicardium, and thus may not reflect ISF adenosine concentration in the deeper myocardial layers, especially in the subendocardium. This may not be a problem since it has been shown that under normal conditions (66) and during aortic constriction (67) adenosine is distributed uniformlyy through the myocardial wall. However, it is quite possible that different interventions may affect the transmyocardial adenosine distribution, thus rendering pericardial infusate adenosine concentration an inaccurate estimate of the ISF adenosine concentration in the deep myocardial layers. The other problem with the technique is

that endogenous pericardial adenosine content does not attain equilibrium with estimated ISF levels (68). Furthermore, pericardial infusate adenosine concentration can be altered by adenosine uptake. Therefore, pericardial infusate adenosine can be a reliable estimate of ISF adenosine only under steady-state conditions.

It is, thus, clear that none of the techniques used for assessement of the ISF adenosine concentration is perfect and the limitations of each should be kept in mind when different studies are compared.

4.4 Adenosine production with increased cardiac performance

Although it has been shown many times that adenosine release under ischemic conditions parallels the change in coronary flow, it is important to demonstrate that coronary flow changes induced by conditions other than ischemia are also mediated by adenosine.

Weidmeir and Spell (69) studied effects of changes in myocardial oxygen demand (MVO2) and contractility on myocardial adenosine production and coronary flow in isolated guinea pig hearts. They found that catecholamines increased MVO2 and contractility, while adenosine concentration in the coronary sinus blood increased proportionally to the degree of increase in coronary flow. Nitroglycerine, on the other hand, increased contractility but did not change MVO2. No change of adenosine

concentration was detected.

Miller et al. (68) studied the effects of stellate ganglion stimulation on MVO2, coronary flow and adenosine production using pericardial infusate technique. They found that ganglion stimulation resulted in the increase in MVO2, pericardial infusate adenosine and coronary flow to the same extent.

Watkinson et al. (70) using pericardial infusate technique demonstrated increased adenosine production with stellate ganglion stimulation as well as with treadmill exercise. In these experiments, dos were fitted with a tube connected to the pericardial sac. Adenosine levels were measured before, during, and after the treadmill exercise. No difference was found between adenosine levels before and after the exercise, but a 2- to 3-fold increase in the infusate's adenosine concentration was noted during the exercise.

Later, McKenzie et al (67) demonstrated that after 10 minutes of treadmill exercise at 5 mph, there was a 2-fold increase in coronary flow and MVO2 associated with a 3-fold increase in the myocardial adenosine levels.

Saito et al (71) used tissue sampling technique to evaluate changes in adenosine concentration induced by pacing, aortic constriction, or beta-blockade. In all cases, adenosine concentration varied directly with changes in

MVO2, while coronary resistance varied inversely with changes in adenosine's concentration.

Manfredi et al. (64) also studied changes in adenosine concentration, using coronary sinus- arterial blood concentration difference multiplied by coronary plasma flow, MVO2 and coronary resistance during atrial pacing and norepinephrine infusion. They found that during norepinephrine infusion adenosine concentration rose proportionally to the fall in coronary resistance, while MVO2 also rose. But during atrial passing there was observed no change in adenosine concentration as MVO2 rose and coronary resistance fell.

Bacchus et al. (61) studied changes in coronary flow and adenosine concentration by pericardioal infusate method in chronic dog during treadmill exercise, feeding, and exposure to loud noises. They found a significant increase in adenosine's concentration with all interventions, and a significant correlation between MVO2, coronary flow and changes in adenosine concentration.

Thus, a variety of studies seem to indicate that adenosine may be a mediator of coronary flow changes during increased myocardial performance, as caused by treadmill exercise, norepinephrine stimulation, feeding, excitement due to loud noises or stellate ganglion stimulation. Effects of tachycardia are less clear with one group (71) claming that induced changes in coronary flow are adenosine-

mediated, while another group (64) found no association between changes in adenosine's concentration and flow. The difference is most likely related to the differences in techniques and further studies are needed to settle this matter.

Another way of altering coronary blood flow, in addition to ischemia and increased cardiac performance, is nonischemic hypoxia. HF Downey et al. (72) studied the effects of nonischemic hypoxia on coronary flow by selectively perfusing the left anterior descending artery with hypoxic blood (PO2 11.7 torr). They observed a 600% rise in coronary flow, similar in magnitude to the rise in flow observed with ischemic hypoxia (coronary artery occlusion). ISF adenosine concentrations were measured by myocardial sampling technique. It was found that the ISF adenosine concentration was actually reduced while coronary flow surged. However, assessement of the ISF adenosine concentration by direct tissue sampling under conditions of nonischemic hypoxia may be especially inaccurate. Indeed, adenosine's metabolism or release has not been studied under such conditions and it is qute possible for ISF adenosine to increase while the total tissue adenosine concentratio is decreased. Furthermore, nonischemic hypoxia is a rather nonphysiologic stimulus, especially as severe a one as was used by Downey et al. (72). Therefore, it is quite possible that coronary flow changes induced in such a way are not mediated by adenosine but rather directly by oxygen.

As is well known, myocardial MVO2 is greater during systole than diatole. In a way, we have a physiologic model of increased cardiac performance during a specific part of the cardiac cycle (systole). Thus, one of the greatest challenges for adenosine hypothesis is to prove that the ISF adenosine concentration can change fast enough to accomodate changing myocardial needs during systole. The answer to this question was given in an elegant study by Thompson et al (73). They studied systolic and diastolic myocardial concentrations of adenosine and its degradative products, inosine and hypoxanthine, in isolated perfused guinea pig hearts. The heart rate was slowed down and the systole and diastole were prolonged by 186% and 907% respectively by crushing the sinus node and substituting Sr for Ca in the perfusate. The authors found a significant increase in adenosine and its degradative products during systole as compared to diastole. The addition of EHNA, an adenosine diaminase blocker, resulted in a significantly higher systolic than diastolic adenosine concentration.

To summarise, we have an impressive array of evidence implicating adenosine as the principal mediator of coronary blood flow during physiologic conditions as well as during conditions of ischemia or increased myocardial performance. Furthermore, it was shown that adenosine concentration can change fast enough to accomodate changing myocardial needs during a single cardiac cycle. Thus, although not all of the criteria set forth by Berne are as yet met by adenosine

it appears increasingly clear that it will prove to be the mediator of coronary blood flow.

4.5 Adenosine metabolism

Some of the aspects of adenosine's metabolism have already been discussed in the section on direct tissue sampling. It was originally assumed that AMP formed as a result of ATP hydrolysis (ATP - ADP - AMP) serves a substrate for 5'- NT, an ecto enzyme, and that almost all adenosine is formed extracellularly (57,74). As has already been mentioned, analysis of breakdown products of prelabeled nucleotide precursors suggests that adenosine may, to a substantial extent, be produced and localised intracellularly (58,59). Schutz et al. (60) found that 5'-NT inhibitor did not markedly reduce the rate of ischemia-induced adenosine production, while infusion of nucleoside transport inhibitor significantly decreased adenosine production. Furthermore, it was found that appreciable quantities of 5'- NT are located in a 200,000- g cytosol fraction (60).

Olsson et al. (75) suggested that AMP used as a substrate for adenosine production is generated from cAMP by the action of phosphodiesterase, rather than from hydrolisis of ATP. The size of adenosine pool would therefore be dependent on the concentration of cAMP and factors that affect it. There is, unfortunately, no hard data to determine whether adenosine is produced via cAMP or ATP

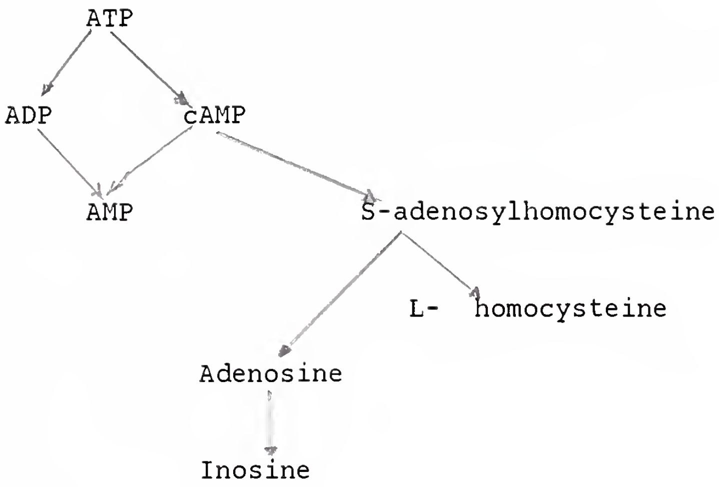
hydrolysis pathway.

Recently, a new possible source of adenosine in the heart has been described. Schutz et al. (60) found that there was a high specific activity of S- adenosylhomocysteine (SAH) hydrolase present in the guinea pig hearts. The enzyme was present exclusively in the cytosol and exhibited a low Km. It catalysis the followong reaction:

SAH = Adenosine + L- homocysteine

Although the equillibrium constant of this reaction favors synthesis of SAH, under conditions prevailing in the myocardium it may not be so.

Adenosine concentration in the interstitial fluid depends on the relative rates of its production, reuptake by myocytes, washout and conversion to inosine and hypoxanthine by adenosine deaminase (46). Conversion to inosine and hypoxanthine appears to be the predominant mechanism. Thus, the following scheme of adenosine metabolism can be proposed:



It remains to be determined whether AMP used in adenosine production is formed from cAMP or ATP hydrolysis, and what role does SAH play in adenosine metabolism. Furthermore, it is necessary to determine how intracellularly formed adenosine is protected from degradation by adenosine deaminase, a ubiquitous cytosol enzyme. Also, adenosine transport across the plasma membrane and stimuli for its release need to be studied.

4.6 Mechanism of action

The molecular mechanism of adenosine's action is not well understood. There exist several possibile mechanisms. One possibility is that adenosine acts via production of an increased smooth muscle cAMP concentration. Increase in cAMP concentration has been shown to be associated with vascular smooth muscle relaxation (76). Furthermore, adenosine has been shown to increase cAMP content in the brain (77). However, Herley et al. (78) found that only pharmacological doses of adenosine resulted in significant

increase in cAMP levels in hog carotid artery strips. Several other investigators also reported essentially similar results (79).

A second possibility is that adenosine acts by inibiting calcium transport in smooth muscle cells or interferes with calcium utilization in the contractile machinery of these cells (57). Adenosine was shown to decrease the force of contraction, uptake of Ca++ and the plateau phase and duration of action potential in atrial muscle in guinea pig (80).

A third possibility is direct adenosine induced relaxation of vascular smooth muscle. Thereis a strong evidence for existence of a specific adenosine receptor on cardiac muscle, coronary arteries and carotid arteries in the dog and rabbit (79). It is unknown at present whether adenosine acts via one or a combination of these mechanisms. It does seem clear that the vasodilator action of adenosine is initiated by the attachment to the coronary myocyte's adenosine receptor (57).

Schrader et al. (81) synthesized large molecular weight protein- AMP conjugate that was confined to the extacellular space. When infused into coronary arteries of isolated guinea pig hearts, it induced vasodilation similar in magnitude and time course to vasodilation elicited by adenosine or AMP. It was concluded that since the observed vasodilation was most likely induced by adenosine moiety of

AMP, there exists a surface receptor for adenosine and/or AMP.

Lee et al. (82) studying binding of a specific adenosine receptor agonist, phenylisopropyladenosine, to an enriched cardiac sarcolemma preparation found that the specific binding was rapid and saturable. Computer analysis revealed a single class of binding sites. Schocken et al. (83) used labeled adenosine to assess its binding to the ventricular myocardium. They found that competetive binding for labelled adenosine was saturable, of high capacity, with extremely rapid reversability, and high affinity. Furthermore, adenosine-induced dilatation of the coronary vessels is not affected by adrenergic blocking agents, indicating that the adenosine receptor is separate from adrenergic receptors (57).

5 Transmyocardial Distribution of Coronary Blood Flow

The studies of transmyocardial distribution of coronary blood flow effectively began with the work of Kirk and Honig (33). They studied the blood flow distribution and oxygen tension in normal canine hearts by depot-clearing method. These studies involve injecting I131-labeled sodium iodide into the myocardium at varying depths and estimating clearance of the isotope over time. The obtained data indicated at least 25 per cent slower blood flow in deep (subendocardial) layers than in superficial regions. Using Xe133 washout studies, Brandi et al. (84) also found relative hypoperfusion of subendocardial layers under basal conditions. On the basis of these and several other studies also employing the depotclearing method (33,84,85), it was suggested that anaerobic metabolism may take place in the subendocardium (33), or that subendocardial layers might be perfused directly from the LV cavity via lumenal channels (86).

Further support of these hypotheses came from studies indicating that relatively higher levels of glycogen, phosphorylase activity (87), and other glycolitic enzymes (88,89) exist in the subendocardium. Allison and Holsinger (90) found significant transmural gradients of high energy phosphate compounds and lactate, which they interpreted as evidence of reduced oxydative and increased glycolytic reserve in the endocardium. Based on these findings, a model

of transmural flow regulation was proposed. This model postulates that endocardial vessels are maximally dilated in order to provide nutrition to the endocardium, which is nearly ischemic even under basal conditions. The endocardium is also relying on anaerobic metabolism to meet its energy requirements (91).

The hypothesis that subendocardial layers are supplied from the LV cavity via lumenal channels was soon shown to be incorrect (92). There is also an impressive array of evidence against predominantly anaerobic metabolism in the subendocardium (89-91).

Further advances in the field occurred with the development of microsphere flow studies. It was conclusively demonstrated that the transmural distribution of 9 micron microspheres closely approximates blood flow (94,95), and that of 15 micron microspheres also quite accurately reflects the flow (96). Microsphere blood flow distribution studies soon demonstrated that there was no underperfusion of the subendocardium under basal conditions, and that in some cases subendocardial flow even exceeded subepicardial flow (97-99). Previous studies utilizing depot-clearing technique of flow determination were further criticised on the ground that local conditions created by isotope injections (local edema, hemorrhage, local vasomotion and leakage of the injectate up the needle tract) distort the flow data (100). It is, therefore, considered established

that subendocardium is not underperfused under normal conditions and that, indeed, transmyocardial flow is distributed equally through the LV wall.

One, thus, needs to account for this equality in distribution, especially considering the much greater systolic extravascular resistance in the subendocardium. As it has been aluded to previuosly, when coronary flow is restricted to systole, the epicardial flow geartly exceeds the endocardial flow. This difference is directly attributable to the systolic transmyocardial pressure gradiet (101). As has been discussed in the section on extravascular resistnce, coronary vessels compression by the contracting myocardium is the predominant factor determining the magnitude of the systolic extravascular resistance, while systolic strains play only a minor role. To expain the uniformity of transmyocardial flow, it is necessary first to discuss the factors regulating transmural flow distribution in systole and in distole.

5.1 Systolic transmural flow distribution

To explain the predominant perfusion of subepicardial over subendocardial areas when the flow is confined to systole, Downey and Kirk (102) proposed a model explaining this gradient. The proposed model, called vascular waterfall model, is based on assumptions somewhat different from the conventional flow dynamics. The classic description is given by Poiseuille's law

$$Q = (Pi - Po) \times 1/R$$

where Pi is inflow, Po outflow pressures and R is resistance. If, however, we have a collapsible vessel surrounded by a higher pressure medium, the situation changes. Now, if the extravascular pressure (Pc) exceeds the outflow pressure, the flow is determined by the extravascular pressure. The flow equation, therefore, changes as follows:

$$Q = (Pi - Pc) \times 1/R$$

A peculiarity of this system is that a change in flow has no influence on the pressure drop across the end of the tube, and a change in the pressure drop across the end of the tube has no influence on flow (103). Resistance in such a system is determined by the interaction of Pi and Pc. More specifically, it is determined by the degree of vessel constriction created by these two opposing pressures. When the constriction created by the partial vessel collapse generated by Pc results in sufficint luminal pressure to prevent further vessel narrowing, an equilibrium will be reached. The cross- sectional area of the orifice (narrowing) created by the vessel constriction will, therefore, determine the flow through the whole system. This can be described by the following equation:

$$Q = A \quad 2 \times (Pc - Pi) \times 1/d$$

where A is cross- sectional area of the orifice and d is density of flow (103). In Downey and Kirks's model coronary circulation consists of numerous parallel channels, each

responding to the local tissue pressure by forming vascular waterfalls. Therefore, higher intramyocardial systolic pressure in subendocardial layers will result in formation of vascular waterfalls there with resulting decrease in subendocardial flow.

It should be noted that the waterfall model can only be applied to systems with very low Reynold's numbers, i.e. < 1 (104). Blood flows characterized by such low Reynold's numbers occur only in the microcirculation and it is not entirely clear if the waterfall model can be accurately applied to the coronary circulation. However, the resulting error, if an, is likely to be rather small.

5.2 Diastolic transmural flow distribution

Thus, it is well established that during systole epicardial flow greatly exceeds endocardial. And yet, the endo/epi flow ratio remains near unity. It follows therefore, that diastole largely favors flow in the subendocardium. The mechanism(s) for this preferential diastolic endocardial flow is unclear.

To account for the equality in the transmural flow distribution, one has to postulate a lower diastolic vascular resistance in the subendocardium than in the subepicardium. Diastolic vascular resistance is determined by the extravascular resistance (diastolic intramyocardial pressure), intravascular resistance (coronary artery tone)

and the gradient of vascularity.

The subject of the diastolic intramyocardial pressures has been discussed at length in the section on extravascular resistance. There exist conflicting results regarding the existence, direction, and magnitude of the diastolic pressure gradient. But even if one assumes higher intramyocardial pressures in the subepicardium than in the subendocardium, the magnitude of pressures themselves is probably not sufficient to account for a much higher diastolic endocardial flow. Thus, while during systole, extravascular resistance plays a dominant role in the transmural flow distribution, during diastole its role is a minor one.

The concept of the gradient of vascularity implies anatomically determined higher vessel density in the endocardium rather than the epicardium. While there are very little direct anatomical data supporting this hypothesis, some phsiological data appear to be consistent with it.

Downey HF et al (105) studied transmyocardial flow distribution in fibrillating hearts with pharmacologically induced maximal vasodilation. They found that under such conditions endo/epi flow ratio was 1.37. This can be interpreted to indicate that the endocardial vascular resistance is 30-40% lower than the epicardial. Downey JM and Kirk (106) studying transmocardial flow distribution in

arrested hearts with maximally dilated coronary arteries found that the endocardial resistance was 20% lower than the epicardial. Wusten et al (107) also found that the endocardial vascular resistance is lower than the epicardial. It appears, therefore, that indeed vascular density is higher in the subendocardium than in the subepicardium.

If we assume that at normal heart rates diastole is about 3 times longer than systole, then 30% difference in resistance due to the gradient of vascularity, aided perhaps by the intramyocardial pressure gradient, would be just enough to equalize the total flow distribution. It is clear, however, that, for example, tachycardia which results in the relative shortening of diastole, would result in endocardial underperfusion if no other mechanism were opertional. Studies performed on chronically instrumented awake dogs demonstrated that when maximal vasodilation was achieved with adenosine, increse in the heart rate resulted in the fall of the endo/epi flow ratio (108). When adenosine was not administered, endo/epi ratio remained constant despite the increase in the heart rate from 100 to 250 beats/min. Therefore, some active vasomotion is required to maintain the uniformity of transmyocardial blood flow during tachycardia as well as during other stresses on the heart.

A variety of metabolic factors such as K+, lactate, CO2, ATP/ADP can conceivably accumulate in the endocardium and be

responsible fr this vasomotion. In most studies, however, there were found no differences in the concentrtion of these metabolites between the epicrdium and the endocardium. (109,110,91). Perhaps, adenosine, which plays a major role in coronary flow regulation, can be responsible for maintaining the equality of transmural perfusion? Studies on transmural adenosine concentration showed no significant difference between the epicardium and the endocardium under normal conditions (66) or during aortic stenosis (67). Coronary artery stenosis, however, resulted in higher endocardial than epicardial adenosine concentration (66). But this degree of coronary artry stenosis (50%) is known to produce transmyocardial lactate gradient which indicates the occurence of subendocardial ischemia (91) and, thus, cannot be considered a fair test of adenosine's role.

No data are available regarding the transmural adenosine concentration during tachycardia or other states of increased cardiac performance. It may not be necessary, however, to have a higher endocardial adenosine concentration in order to obtain a necessary degree of coronary subendocardial vasomotion. The other possibility is that endocardial vessels may be more sensitive to adenosine than epicardial, or, perhaps, a threshold for adenosine induced vasodilation is lower in the subendocardium than the subepicardium.

Rembert et al.(111) found that submaximal doses of

adenosine resulted in the increased endo/epi flow ratio, wich could not be explained by the difference in PO2, since even when PO2 was unphysiologically high, the increased endo/epi flow ratio still persisted. There were, however, significant differences in the heart rate, and systolic and diastolic blood pressures between the control, maximal vasodilation, and and submaximal vasodilation groups, all of wich could influence the transmyocardial flow distribution. On the other hand Warltier et al. (112) using isolated canine hearts found that intracoronary infusuion of adenosine produced a dose- dependent shift in transmural coronary flow away from the subendocardium.

To further investigate the role of adenosine in the transmyocardil flow distribution and the question of higher endocrdial vessel sensitivity to adenosine, we studied coronary flow changes under conditions of varying adenosine concentration. Obtained results (113) indicate that, indeed, in the absence of heart rate, blood pressure, and left atrial pressure variations, submaximal doses of adenosine increse preferentially subendocardial blood flow, resulting in the increased endo/epi flow ratio.

Maximal vasodilation induced by adenosine in the beating hearts has been shown on many occasions to result in decreased endo/epi flow ratio. This is not surprising, considering that large doses of adenosine administered to achieve this maximal vasodilation virtually eliminate any

subendocardial vasomotion. Then, the sytolic extracoronary resistance becomes the only remaining factor influencing the flow distribution.

On the basis of the described studies, the following model of the transmural flow regulation can be proposed. During systole vascular waterfalls are created which result in significantly lower flow to the subendocardial than subepicardial areas. During diastole several factors contribute to maintence of the adequate perfusion. First, the gradient of vascularity favoring the endocardium exists in the myocardial wall. This gradient is of sufficient magnitude to assure adequate endocardial flow at normal heart rates and MVO2. Endocardial vessels are more sensitive to adenosine than epicardial vessels, and this adenosine-induced vasomotion accounts for the uniform transmural flow distribution during tachycardia or states with increased myocardial performance.

Obviously, further studies are needed to substantiate this model. Thus direct tests of coronary arteries from the endocardial and epicardial areas can give additional information regarding the differences in adenosine sensitivity. It has been shown that adenosine concentration can change fast enough to accomodate for the difference in MVO2 between systole and diastole (73). It is, however, unknown if its concentration can change fast enough at rapid heart rates. Finally, the prescise nature of transmyocardial

diastolic pressure gradient and its contribution to the diastolic extravascular resistance should be elucidated.

CHAPTER TWO

CATECHOLAMINE CARDIOMYOPATHY

1 Introduction

Catecholamines given in high concentrations produce considerable myocardial damage. This has been demonstrated experimentally in several mammalian species (114-116). Similar changes have been found in patients dying of shock who were given large amounts of catecholamines (117,118) and in patients with pheochromocytomas (117, 119-121).

The histopathological changes induced by catecholamines include myofiber necrosis, myofibrillar degeneration and leukocytic infiltration (114-116). The lesions are usually more prominent in the subendocardial areas. It has been demonstrated that the severity of the injury is a function of both the dose and duration of norepinephrine (NE) infusion (116,122). A reproducible model of NE cardiomyopathy in rabbits has been developed by Downing and Lee (116). They showed that a short-term (90 minute) infusion of NE in relatively modest doses (2-3 g/kg/min) leads to a consistent pattern of myocardial injury, readily identifiable by light microscopy as well as by radionuclide cardiac imaging (116,123).

Studies of cardiac function utilizing either afterload curves (124) or standard VF curves (125) revealed

significant impairment of LV performance. Thus, it has been conclusively established that administration of NE leads to substantial myocardial injury associated with reduced LV performance. The pathogenesis of this cardiomyopathy has, not been clearly established, however.

It has been shown recently that while beta-blockers do not prevent NE-induced damage, alpha-antagonists such as phentolamine can effectively prevent the lesion (126). Furthermore, administration of a pure alpha-agonist such as methoxamine, results in essentially identical lesions, and the severity of these lesions depends on the dose of methoxamine given (126). This suggests the possibility that the mechanism of lesion production in the norepinephrine cardiomyopathy involves coronary vasoconstriction through alpha-adrenergic pathways, sufficient to cause injury to the myocardium. The present study was undertaken to test this hypothesis.

2 Pathology

The cardiotoxic properties of catecholamines have been known for a considerable length of time. Pearce in 1906 was one of the first to describe myofibrillar edema, loss of striation and increase in fibrous tissue in the heart after several injections of adrenaline (124). Soon thereafter, it was shown that norepinephrine can also induce a cardiomyopathy (125). Since than, cathecholamine cardiomyopathy has been demonstrated to occur in several species of laboratory animals, including rabbits, dogs and cats (114-116). It has also been reported to occur in people treated with large doses of catecholamines as well as in patients with pheochromocytomas (117, 119-121).

Morphologically, the lesions of catecholamine cardiomyopathy are similar in all species examined, including man, and do not vary with the catecholamine used. The severity of the injury depends on the dose and duration of administration. The gross and microscopic appearance of the lesions depends on the time elapsed between the dose administration and the sacrifice of the animal.

Early changes include subendocardial congestion and hemorrhage (118,122,128,129). Occasionally, edema and hemorrhages into the valve leaflets are also present (128). On microscopic examination, vascular congestion, interstitial edema and focal myofiber degeneration with loss of individual myofiber striation are observed (118,128,130).

Vacuolization and accumulation of the lipid material in the myocardial cells are also commonly seen (118,130). The first changes to be detected under electronmicroscopic examination, minutes after catecholamine infusion, are areas of hypercontracted myofibrills with wide Z lines and "contraction bands" (130,131). Tearing of myofilaments away from sarcomeres has also been noted (131). Other observations include reduced numbers of glycogen granules and dilatation of the transverse tubules (130,132).

Within several hours myocardium appear stiffened and less pliable (133). Microscopically, interstiatial edema with polymorphonueclear cell infiltration is observed. Electronmicroscopically, the number and extent of "contraction bands" increases and the sarcomeres begin to show loss of architecture (114,131). Somewhat later, mononuclear cells appear and gradually come to predominate in the lesions. Perivascular Anitschkow myocyte type cells are also observed. Fibrinoid degeneration of arterioles has also been noted (118,122).

Two to five days later, areas of fibrosis can be observed on gross examination. Microscopically, foci of necrosis with dissolution of myofibers and infiltration of mononuclear cells are seen. At this time also, scarring appears in the interstitium. Depending on the dose and duration of drug administration, the lesions are either transmural or subendocardial. But even with transmural

injury, subendocardial layers are affected to a greater extent than are subepicardial layers. Over the next several weeks, the acute inflammatory changes subside and fibrous replacement of the destroyed myocardium occurs.

3 Pathophysiology

The catecholamines produce a rather specific morphological pattern of injury. However, it is important to determine if this damage has physiological consequences such that the functional capacity of the heart is reduced. Werner et al. (124) using a rabbit model of NE induced cardiomyopathy studied the effects of changes in afterload on ventricular function. They found that in cardiomyopathic rabbits pressure-function curves were lower than in control animals, indicating reduced LV performance.

Myocardial contractility in animals with catecholamine cardiomyopathy was studied by Fripp et al. (134) They found that resting dP/dt max did not differ from control animals, suggesting no difference in the resting contractile state. However, proportional increases in dP/dt max during NE administration were sharply lower in cardiomyopathic than in control animals. Because the cardiomyopathic animals exhibited some evidence of left ventricular failure, as manifested by increased LVEDP, a reduction in intramyocardial NE stores and increased sensitivity to exogenous NE would be expected. Since NE infusion studies demonstrated decreased sensitivity of the myocardium to exogenous NE administration, changes in contractility in this case are probably not related to altered myocardial NE stores. When contractility was assessed during infusion of Ca++, it was also found that the rise of dP/dt max in

cardiomyopathic group was much smaller than in control animals. This suggests some abnormality in the contractile apparatus itself, possibly in excitation-contraction coupling (134). Since the response to Ca++ was also diminished, the beta-receptor system was probably not involved.

The studies discussed above have demonstrated that catecholamine cardiomyopathy results in decreased capacity to respond to afterload stress (124), and reduced inotropic responses to NE and Ca++ administration. The latter finding indicate reduced ability to augment contractility (134). It is well known, however, that changes in contractility may dissociate from changes in the pump function in several pathophysiological states (135,136). Hence, it is important to directly examine pump function in catecolamine cardiomyopathy.

Studies of left ventricular function using changes in preload demonstrated a significant reduction of the pump function. Mean stroke volume at LVEDP of 10 cm H2O (SV10) was 1.16 ml in the control group and .93 ml in the cardiomyopathic animals, a 20% reduction (125). On the other hand, the ability of the cardiomyopathic hearts to augment the global pump function was the same as that of control animals as demonstrated by a comparable percent increase in SV10 during catecholamine administration (125). Thus catecholamine cardiomyopathy is yet another state (in

addition to circulatory shock and myocardial infarction), where there exists a dissociation between the global pump function and contractility.

4 Pathogenesis

A number of theories have been advanced to explain the mechanism of catecholamine induced damage. As has been noted, one of the earliest morphological features is subendocardial hemorrhage. Since vagal stimulation has also been shown to result in subendocardial hemorrhages, and since catecholamine administration results in increased vagal discharge, Szakacs (117) suggested that provoked vagal discharge is at least partially responsible for the observed injury.

Catecholamine induced alterations in lipid metabolism has been proposed as another possible mechanism. Hoak (130) noted that catecholamines stimulate mobilization of lipids from the adipose tissue stores, producing increased levels of serum free fatty acids (FFA). This could result in increased myocardial FFA concentration with uncoupling of oxidative phosphorylation and direct myocardial tissue damage. There are, however, a number of problems with this hypothesis. First, it is known that the lesions of catecholamine cardiomyopathy predominate in the subendocardium (118,122). It is not clear how the lipid hypothesis would account for such a distribution. Secondly, myocardium possesses very efficient means for matabolizing free fatty acids. Furthermore, it remains to be demonstrated that increased plasma levels of FFA result in increased myocardial FFA concentrations or that increased

myocardial FFA content results in myocardial damage.

Haft and colleagues proposed that occlusion of small cardiac vessels by platelet aggregates is the dominant mechanism of damage in catecholamine cardiomyopathy (137-139). When dogs were pretreated with aspirin or dipyridamole prior to epinephrine infusion, the extent of injury was significantly reduced (137). Electronmicroscopic studies immediately after a 4-hr infusion of NE demonstrated platelet aggregates in the small vessels (138). It was postulated that platelet thrombi play a significant role in catecholamine injury (139). However, other investigators have been unable to confirm the presence of platelet aggregates after catecholamine administration (121,126). Platelet microthrombi hypothesis also does not explain the subendocardial predominance of the lesions. While it is not clear why pretreatment with dipyridamole or aspirin resulted in decreased injury it is unlikely that platelet aggregates play a substantial role in the development of catecholamine-induced damage.

As early as 1909 Fleisher and Loeb suggested that violent contractions caused by adrenaline may result in myocardial damage (133). Nahas (129) studying myocardial effects of catecholamines in an isolated heart-lung preparation, suggested that a prolonged myocardial tension may be responsible for the lesion production. Csapo et al. (131) studying early electronmicroscopic changes after

isoproterinol administration found that the first observed changes were related to hypercontraction of myofilaments with formation of contraction bands and disorganized and fragmented myofibrills.

Pretreatment with insulin sharply reduces the inotropic response of the muscle to NE in the isolated heart (140). When insulin is administered to intact animals before NE infusion, the extent of the damage is substantially reduced (116). These data support a mechanical injury hypothesis, indicating that reduced inotropic responsiveness after pretreatment with insulin reduces catecholamine induced damage.

NE is known to increase intracellular cAMP and Na+ concentrations (141,142). Increased intracellular Na+ levels lead to increases in intracellular Ca++ concentrations. This could result either from increased Na/Ca exchange or from release of Ca++ from intracellular stores (143,144). It is well known that elevated intracellular Ca++ concentrations increase inotropic state due to Ca-mediated inactivation of troponin-tropomyosin system and enhanced actin-myosin interaction. Excessive intracellular Ca++ concentrations could conceivably lead to irreversible hypercontraction (116), consistent with the morphological changes described by Csapo et al. (131). It is not clear how insulin prevents the catechol induced damage. Possibly, it limits Na+ influx into the cells by

blocking an insulin-sensitive Na+ pump (145). However, a direct, steroid-like membrane stabilizing effect cannot be excluded (116).

Myocardial tension development is greatest in the subendocardium. Thus myocardium in this region would be expected to be undergo more systolic stretch than in the subepicardium, resulting in increased diastolic sarcomere length. Since it appears that the sarcomere sensitivity to calcium is lengthdependent (146,147), subendocardial myocardium would be more sensitive to changes in the intracellular Ca++ concentration. Hence, most of the damage would be expected to occur in the subendocardial area, as indeed is observed morphologically.

Ischemia is another potential mechanism by which catecholamine damage can be induced. It can result from the vasoconstriction with reduced oxygen supply, or from the increased myocardial oxygen demand disproportionate to changes in coronary flow. As early as 1906, Pearce (127) suggested that adrenalin induced vasoconstriction is responsible for the observed injury. Handforth (148) infused india ink into the coronary arteries of hamsters sacrificed shortly after isoproterinol infusion and found reduced flow to the subendocardial areas of the left ventricle. He concluded that muscle injury was due to the ischemic infarction. Using electronmicroscopic techniques, Ferrans et al. (114) also concluded that isoproterinol

injury is ischemic in nature. Schenk and Moss (122) studying NE induced damage concurred that ischemia is a major mechanism of injury and suggested that a fall in blood pressure observed shortly after the onset of infusion further compromises coronary flow by reducing the perfusion pressure. Because of high systolic tension, subendocardial 02 usage is greater than in the rest of the myocardium. At the same time, subendocardial vascular reserve is less than subepicardial. A combination of these factors makes subendocardium particularly sensitive to reductions in coronary blood flow. Thus any agent inducing myocardial damage by altering coronary flow would be expected to have its greatest impact on the subendocardium, as indeed is observed in catecholamine cardiomyopathy.

Since norepinephrine possesses both alpha and beta adrenergic properties, both pathways may be involved in production of the lesions. Although beta-receptors are clearly the dominant receptors in cardiac tissue (149,150), a recent study failed to show any protective effect of beta-blockade with large doses of either propranolol or proctolol on NE-induced damage (126). While this does not rule out a role for beta-receptors in mediating the damage, it is clearly not the predominant pathway. On the contrary, alpha blockade with phentolamine sharply reduces the extent of NE damage (126). Furthermore, lesions can be induced by a pure alpha-agonist, methoxamine, which morphologically are identical to NE-induced lesions. These are also completely

prevented by phentolamine (126). Thus it appears that activation of alpha-receptors is the primary pathway mediating catecholamine injury.

There is substantial evidence regarding the existence of alpha receptors in the myocardium as well as in the coronary arteries (150), the precise mechasnism by which alpha activation contributes to lesion formation is unclear. Myocardial alha-receptor stimulation is known to result in augmented inotropic state (151) as well as in vasoconstriction (152). This again raises the possibility of mechanical damage as well as ischemic injury secondary to a supply-demand mismatch involving increased MVO2 and vasoconstriction. Vatner et al. (152) demonstrated that NE infusion in conscious dogs results in elevated coronary resistance, which is prevented by alpha blockade. Therefore, NE may cause the myofiber damage by altering coronary blood flow. The present study was designed to test this hypothesis and to assess the dynamics of coronary flow and resistance changes during a NE infusion.

CHAPTER THREE

NOREPINEPHRINE INDUCED CARDIOMYOPATHY

1 Methods

Coronary blood flow data were obtained from 15 New Zealand white rabbits. All animals were anesthesized with phenobarbital, 30 mg/kg, intubated and placed on a Harvard respirator. Arterial blood samples were obtained at frequent intervals and appropriate adjustments were made to maintain pH and PCO2 at physiologic values (pH - 7.34-7.44, PCO2 - 34-44 mmHg and PO2 above 85 mmHg) throughout each experiment. Polyethylene catheters were placed in a femoral artery and vein. Arterial pressure was continuously measured with a Sanborn transducer and heart rate was determined with a Sanborn cardiotachometer. The heart rate was verified by manual assessement of pulse frequency from pressure traces inscribed by a Sanborn (Model 358) recorder. Body temperature was maintainerd with a heating pad. A median thoracotomy was performed and the heart was exposed and suspended in a pericardial cradle. A polyethylene catheter was placed into the left atrium for injection of radioactive microspheres. A femoral arterial line was used for collecting an arterial reference blood during microsphere injection.

Coronary blood flow was assessed by administration of differently labeled radioactive microspheres at specified

times during the experiment. Approximately 800,000 microspheres, 15 μ each, labeled with either Ce-141, Ru-103, or Sc-46 (New England Nuclear) were injected into the left atrium. Prior to each microsphere injection the mixture was ultrasonicated and a Harvard withdrawl pump was started (7.5 ml/min) to collect a reference blood sample. This was continued for two minutes after each injection. Upon completion of the experiment, the heart was excized and epicardial fat, blood vessels and atria were dissected away. Each heart was cut into three or four concentric rings in a plane parallel to the atrioventricular groove, and each ring was then cut into two transmural segments. Each transmural segment was then divided into epicardial and endocardial halves. All tissue and reference blood samples were counted in a gamma well counter (Beckman Gamma 8000). The net activity of each isotope was obtained by conventional correction for background, energy overlap, and decay due to counting. Isotopic activity from the arterial reference blood was used to calculate blood flow (ml/mg/min) for each myocardial tissue sample.

An additional group of 10 animals was used to evaluate the degree of damage histologically. Each animal in this group has received a 90-minute infusion of norepinephrine with or without pretreatment with phentolamine. 48 hours later animals were sacrificed and several transmural myocardial sections were obtained for histological study. A previously developed grading scale (116) was used (0 - no

damage; 0.5 - injury limited to subendocardium; 1 - injury affecting one half of the wall thickness; 1.5 - almost transmural injury sparring subepicardium; 2 - transmural injury). Each histological section was read by two independent observes, each observer assigning a score to each section. The scores were avereged and a mean score of the two observes was assigned to each animal.

All results were expressed as mean +/- standard error. All hemodynamic and flow data were evaluated by analysis of variance. A Bonferroni correction (153) for multiple comparisons was made to evaluate significance. The Student t test (154) was used when two groups of unpaired data were compared. Difference was considered significant when $P < 0.05$

Protocol

Norepinephrine (Levophed, Winthrop) was freshly prepared by dilution in normal saline to concentration which provided the dose of 3 μg/kg/min when infused at constant rate of 0.382 ml/min. In a group of 5 animals (Group 1) baseline coronary flow was obtained with injection of Ce-141 microspheres. An i.v. infusion of norepinephrine was started and two additional sets of microspheres (Ru-103 and Sc-46) were injected at 3 and 10 minutes respectively after the beginning of norepinephrine infusion.

In another group of 5 animals (Group 2) a similar protocol was utilized except that after obtaining baseline

coronary flow, another set of microspheres was injected 40 minutes after the norepinephrine infusion was started.

An additional group of 3 animals (Group 3) was given 10 mg of phentolamine (Regitine, Ciba-Geigy) i.v. over two minutes, after the baseline coronary flow was obtained. Norepinephrine infusion was started 10 minutes after the phentolamine injection, and two more sets of microspheres were injected 20 and 40 minutes after beginning the norepinephrine infusion.

In two more animals (Group 4), 0.9% saline was infused i.v. and the radioactive microspheres were infused at 0, 20, 40 minutes after the beginning of saline infusion.

Two additional groups of 5 animals were used to morphologically evaluate the degree of induced injury. 5 animals (Group 5) received 90-minute NE infusion (3 μg/kg/min). Another 5 animals (Group 6) received a similar 90-minute NE after pretreatment with phentolamine (10 mg).

Substantial alterations in arterial pressure and heart rate occured during norepinephrine administration. Maximal changes and integrated mean values were obtained at frequent intervals. The pressure rate product was calculated as an index of metabolic demand.

2 RESULTS

2.1 Hemodynamic data

Arterial pressure and heart rate changes that occured at different time intervals during the infusion of NE (3 µg/kg/min) alone or with phentolamine (10 mg) are summarized in Table 1. Initial values for blood pressure avereged 104 + 5.8 mmHg in Group 1 animals and 92 + 5.6 mmHg in Group 2 animals. Initial heart rates were 271 + 14 and 268 + 6 respectively. Three minutes after the NE infusion was started, the blood pressure rose sharply and heart rate slowed. With continuous infusion of NE, blood pressure gradually declined and heart rate increased to baseline levels. At no point did the pressure-rate product exceed the baseline values and differences did not reach statistical significance (Fig.1). Thus excessive metabolic demand was not a significant factor in the development of catecholamine cardiomyopathy.

In the group of animals given phentolamine before the NE infusion was started (Group 3), there was no change in the heart rate or the blood pressure throughout the infusion (Table 1). The pressure-rate product remained nearly constant (Fig.2).

In two control animals infused with saline for 40 minutes (Group 4) no significant changes in heart rate or blood

pressure occured during the time of infusion (Table 1).

2.2 Blood flow data

Coronary blood flow data and calculated coronary resistance data for all animals are summarized in Table 2. Coronary flow was initially 2.66 cc/gm/min and rose to 3.46 cc/gm/min three minutes after the NE infusion was started. By ten minutes of infusion, flow was back to baseline values. At 40 miunutes flow was 1.51 cc/gm/min, 43% lower than baseline value ($p< 0.01$) (Fig 3). Calculated coronary resistance was slightly, but not significantly elevated three minutes after the beginning of NE infusion. At ten minutes, coronary resistance was significantly higher than baseline. By 40 minutes it had increased by 83% ($p<0.01$) (Fig 4).

Phentolamine given before the beginning of NE infusion prevented both the decline in coronary blood flow and the rise in the calculated coronary vascular resistance. Fig 5 summarizes coronary flow and Fig 6 coronary resistance data in these animals. Thus, alpha blockade with phentolamine prevented any significant changes in either coronary flow or coronary resistance. The time course of coronary flow changes is summarized in Fig 7, and coronary resistance in Fig. 8.

Saline controls showed no change in either coronary flow

or coronary resistance throughout the infusion (Table 2).

2.3 Histological data

Although the data presented above demonstrates that pretreatment with phentolamine prevents NE-induced decline in coronary flow and rise in calculated coronary vascular resistance, it is still necessary to show that phentolamine reduces the severity of NE-induced histological injury. Histological damage scores in phentolamine-pretreated or not pretreated animals are presented in Fig 9. The mean injury score in Group 5 (NE alone) animals was 1.9. Pretreatment with phentolamine resulted in marked reduction of the mean injury score to 0.5 ($p<0.01$). Fig 10 demonstrates a typical histological section from a Group 5 animal. Severe myocardial necrosis with mononuclear cell infiltrate and extensive vacuoalizations are evident. These lesions were widespread and involved the entire thickness of the myocardium. In contrast to this is a histological section in Fig 11, typical of Group 6 (phentolamine pretreated) animals. Here the injury is limited to subendocardium with less extensive necrosis cellular infiltrate and vacuolization than in Fig 9. Fig 12 shows a border zone between the injured and undamaged myocardium. Thus, alpha blockade resulted in marked reduction in the degree of histological damage produced by NE administration.

2.4 Critique of technique

In order to obtain meaningful blood flow estimates using radioactive microspheres certain conditions should be met. These include an adequate sample weight, good microsphere mixing and stable microsphere activity in the blood at the time when the reference blood sample is obtained. The volume of the reference blood sample should be small in comparison to the total animal blood volume so that its withdrawl does not result in acute hemodynamic changes. All these conditions are easily met in dogs, the species for which the microsphere technique was first developed. The rabbit model, however, presents a more difficult problem. First of all, the heart size is small, and only a few pieces (6-8) of an acceptable weight can be obtained. Hence, no meaningful separation into endo- and epicardial flow components could be done. Secondly, the small volume of the left atrium requires that the volume of administered microspheres also be small. Thish can create problems with microspheres precipitating in the injection syringe. Thirdly, a total of 45 cc are needed for three reference blood samples. This amount constitutes a significant proportion of the total blood volume. However, if the bloof withdrawl is continued over two minutes, and an equal amount of warm saline is infused back into the animal after the withdrawl of the first sample, no significant hemodynamic changes occur. Data from control animals infused with saline confirms that the technique does not alter the

magnitude of coronary flow.

5 DISCUSSION

The results of this study indicate that continuous infusion of NE into the rabbit caused a gradual increase of calculated coronary resistance, which became significant 10 minutes after beginning the infusion. This persisted for as long as the infusion was continued. In contrast to the steady rise in resistance, coronary flow behaved somewhat differently. At first, a substantial rise in flow was observed which coincided with an increase in the coronary perfusion pressure. At this time coronary resistance was esentially unchanged. As the infusion was continued, blood pressure and, hence, coronary perfusion pressure gradually declined, while coronary resistance increased. After 10 minutes of infusion, coronary flow returned to normal, and by 40 minutes was significantly reduced.

Myocardial oxygen demand (MVO2) as assessed by the pressure-rate product (PxR) remained unchanged throughout the experiments. The PxR product has been shown to accurately reflect changes in MVO2 under a variety of experimental conditions and interventions (155). In a recent study of MVO2 consumption parameters in closed ches dogs different indices were tested under uniform conditions with maximal variations in hemodynamics and MVO2. In this study the PxR product was found to have a correlation coefficient of 0.86 when compared to direct measurements of MVO2 (155). Hence this measure can be considered a valid measure of MVO2

even under extreme circumstances. Since this parameter remained stable throughout these experiments it is unlikely that excessive metabolic demand was a significant factor in the observed coronary blood flow changes.

It has been recently demonstrated that pretreatment of animals with the alpha blocker, phentolamine, prevented the development of NE induced damage (126). Furthermore, phentolamine prevented a pure alpha agonost, methoxamine, from inducing the lesions (126). Thus, it appears likely that the lesions of catecholamine cardiomyopathy develop as a result of activation of the alpha adrenergic system.

There exists compelling data regarding the presence of alpha receptors on myocytes as well as smooth muscle cells of the coronary arteries. Das and Parratt (156) demonstrated that infusion of phentolamine in cats resulted in increased heart rate, cardiac output, and dP/dt max, while pretreatment with reserpine prevented these changes. Dairman et al. (157) demonstrated increased synthesis and release of cardiac NE caused by phentolamine administration, presumably due to phentolamine blockade of alpha-2 receptors (158).

Methoxamine has been shown to induce positive inotropic effects producing dose-related increase in force development in cat right ventricular papillary muscle (159). This agent has been also shown to result in significantly increased dP/dt max in lambs (160). Furthemore, alpha receptors have

been demonstrated by direct binding studies in rat (161,162) and guinea pig myocardium (163).

In view of these findings one possible mechanism of catecholamine injury would be overactivation of the myocardial alpha adrenergic system resulting in "mechanical" damage. This mechanism would be consistent with a direct damage hypothesis discussed previously.

But on the other hand, alpha receptors have been demonstrated in coronary arteries (164), and several investigators have described coronary flow and resistance changes resulting from NE administration. Thus Vatner et al. (152) in studies on conscious dogs found that administration of NE (1 μg/kg) resulted in an initial drop in coronary resistance, followed one minute later by a significant rise. Alpha blockade with phentolamine prevented the late vasoconstriction and the initial vasodilation was not observed in anesthetized animals. Warltier et al. (165) using isolated dog heart preparation in which total coronary flow was maintained constant, found that administration of NE (2 g/kg) resulted in reduced subendocardial flow and a drop in the endo/epi flow ratio.

Kelley and Feigl (166) admistered intracoronary boluses of NE to anesthetized open chest dogs. They found an increase in total coronary resistance, of which 60% resulted from constriction of the large coronary vessels. To confirm that alpha adrenergic stimulation results in constriction of

both large and small coronary vessels, Vatner et al. studied eight consciuos dogs instrumented with ultrasound crystals around major coronary arteries. Administration of methoxamine (50 μg/kg) to these animals resulted in a 92% rise in calculated total coronary resistance. A similar rise (108%) was observed in large vessel resistance, confirming the fact that alpha-mediated vasoconstriction occurs throughout the coronary bed (167).

Similar effects of NE on coronary flow have also been observed in humans. Administration of tyramine to six patients resulted in increased plasma levels of NE, while plasma levels of epinephrine remained stable. In 5 out of 6 patient a rise in the calculated coronary resistance and a concomitant drop in coronary blood flow was observed (168). In all patients the PxR product remained constant.

These data indicate that NE is capable of considerably influencing coronary flow and resistance. Thus, it is possible that catecholamine cardiomyopathy is induced by changes in coronary flow (see ischemic hypothesis in the previous section). However, it remained to be demonstrated that coronary flow changes induced by NE are of sufficient magnitude and duration to result in changes observed in cardiomyopathic hearts. Furthermore, since phentolamine blockade in known to prevent the lesion production, it should also prevent changes in coronary blood flow and resistance if these changes are to be considered of

pathogenic significance. The results of this study support this hypothesis. First it was demostrated that NE given in concentrations known to result in morphological damage produced a significant fall in coronary flow and increased coronary resistance. Both of these changes were observed as late as 40 minutes after the beginning of NE infusion. Phentolamine given prior to the beginning of NE infusion prevented changes in both coronary resistance and flow.

On the basis of these findings it appears likely that NE induces damage, at least in part, by altering coronary blood flow. It should be pointed out, however, that this study does not rule out the possibility that some of the damage is induced by alterting the inotropic state of the myocardium. Quite possibly both of these mechanisms contribute.

As has been noted in the discussion of pathology, lesions of catecholamine cardiomyopathy tend to predominate in the subendocardium. It is reasonable to expect any substance affecting coronary blood flow to cause the most damage in the subendocardium, as is observed with NE infusion. However, it is also pssible that NE is itself capable of altering the endo/epi flow ratio. The data in this regard are conflicting. Warltier et al. (165) observed a decline in the endocardial flow and the endo/epi flow ratio in isolated dog heart preparation in which the total coronary flow was maintained constant. On the other hand, Dumount and Lelorier (169) using acute dogs observed an insignifi-

cant rise in the endo/epi flow ratio. Bolli et al. (170) studying methoxamine infusion in acute dogs observed a rise in the endo/epi flow ratio. As has been noted in the section on Results, the technical limitations of the rabbit model did not allow a separate dermination of endocardial and epicardial coronary flow components. This matter, therefore, will require further work.

It is interesting to speculate on how NE alters coronary flow and whether alpha mediated vasoconstriction is the only mechanism involved. As has been discussed in the section on coronary flow regulation, adenosine appears to be the major metabolite responsible for active coronary vasomotion. Adenosine is known to antagonise catecholamine effects on the myocardium. Thus, adenosine administration decreases isoproterenol induced rises in cAMP, protein kinase and phosphorylase in myocytes (171,172). It has also been shown to decrease dP/dt max and rate of ventricular relaxation (172). Thus adenosine may share a common receptor(s) with NE.

It is also known that catecholamine administration results in increased release of adenosine from the myocardium. This effect is short-lived, however. In studies on isolated guinea pig hearts, norepinephrine infusion resulted in increased adenosine release which peaked at four minutes. After ten minutes of infusion, adenosine levels declined (173). This time course of adenosine concentration

changes corresponds closely to changes in coronary flow observed in this study where a considerable rise was observed at 3 minutes with flow returning to normal by 10 minutes. It can be speculated that NE-adenosine interaction is responsible for the flow changes during the first ten minutes of catecholamine administration. Later, as the protective effect of adenosine wears off, alpha-mediated vasoconstriction becomes predominant and coronary flow falls.

In summary, this study has demonstrated that NE administration in doses known to induce typical catecholamine cardiomyopathy, results in substantial alterations of both coronary blood flow and resistance. Myocardial oxygen demanded as assessed by the PxR product remained unchanged and probably did not play any pathogenetic role. Administration of an alpha blocker, phentolamine, prevented changes in both coronary flow and resistance. It is concluded that NE causes myocardial injury in part by decreasing coronary blood flow due to induced vasoconstriction. It is possible that adenosine temporarily opposes these effects of NE. But if the NE infusion is continued for a sufficient length of time, alpha-mediated vasoconstriction becomes predominant and the ischemic damage results.

Table 1

Arterial blood pressure and heart rate values before and during infusion of agents indicated

	Norepinephrine (NE)				NE + Ph (Group II)				Saline (Group III)			
	T	HR	BP	PXR	0	HR	BP	PXR	T	HR	BP	PXR
Group I (N=5)	0	271	104	28,120	0	287	113	32,550	0	275	105	28,000
		±14	±5.8	±1,922		±12	±16.9	±5,449		±25	±5	±3,000
	3	200	141	28,000	20	287	99	28,460	20	275	96	26,365
		±17	±6.2	±2,042		±3	±12.2	±3,790		±25	±1	±3,085
	10	245	113	27,744	40	273	103	28,367	40	275	85	23,325
		±15	±3.0	±1,961		±7	±9.3	±3,159		±25	±2	±1,575
Group Ia (N=5)	0	268	92	23,304								
		±6	±5.6	±1,138								
	40	222	110	24,212								
		±10	±7.6	±1,582								

T, time (min); HR, heart rate (bpm); BP, arterial pressure (mmHg); PXR, pressure-rate product (units), Ph, phentolamine (10mg). (Mean, ± SE).

Table 2

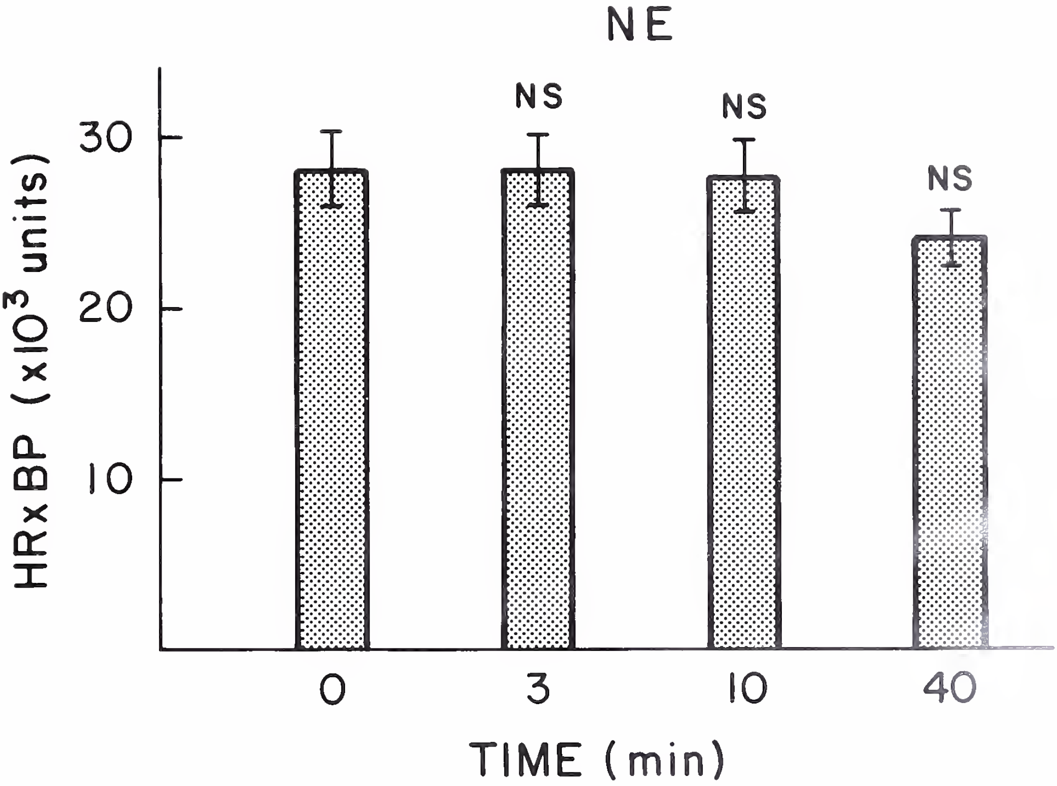
Coronary blood flow and resistance in response to various agents indicated.

Norepinephrine			NE + Ph			Saline		
T	CBF	CR	T	CBF	CR	T	CBF	CR
0	2.66	40.9	0	2.66	43.7	0	2.69	39.2
	±.23	±2.6		±.18	±2.6		±.07	±2.8
3	3.46*	43.1	20	2.94	35.4	20	2.58	37.3
	±.07	±1.5		±.30	±2.4		±.07	±.7
10	2.33	50.5*	40	2.24	50.0	40	2.69	33.3
	±.15	±2.8		±.33	±4.6		±0.9	±3.3
40	1.51*	74.8*						
	±.12	±9.1						

*$P < 0.05$

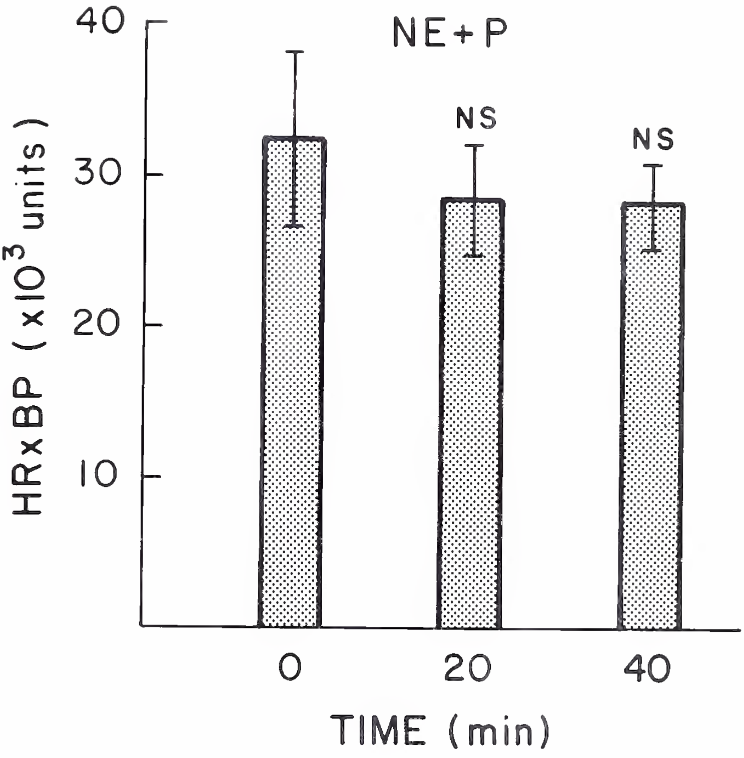
T, time (min); CBF, Coronary blood flow (ml/min/gm); CR, coronary resistance (mmHg/ml/min); Ph, phentolamine (10 mg).

FIGURE 1



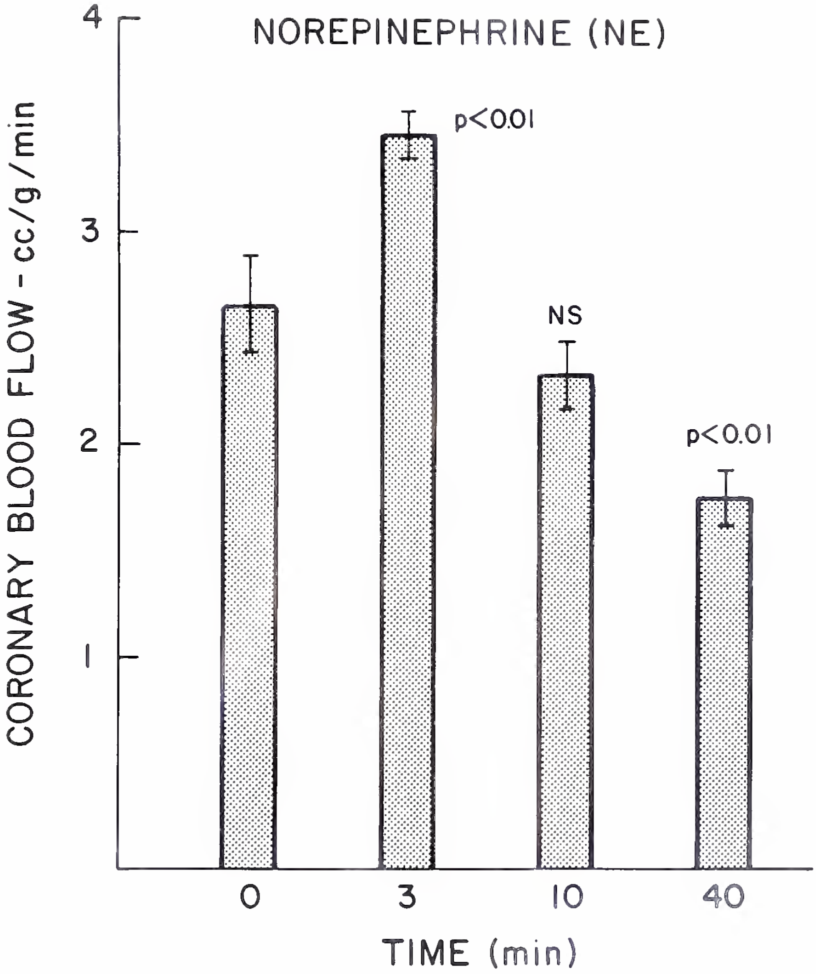
Heart rate (HR) - blood pressure (BP) product (units) measured at four time intervals in animals infused with norepinephrine (NE). Vertical brackets indicate SEM.

FIGURE 2



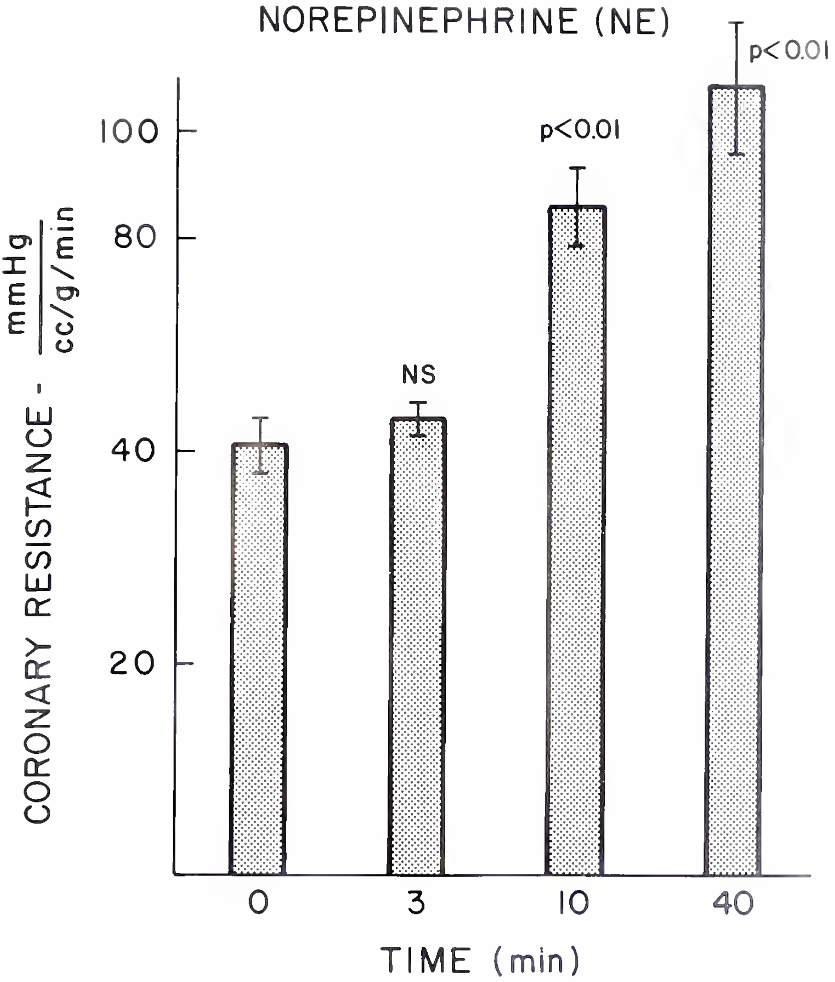
Heart rate (HR) - blood pressure (BP) product (units) measured at three time intervals in phentolamine (Ph) pretreated animals. Vertical brackets, SEM.

FIGURE 3



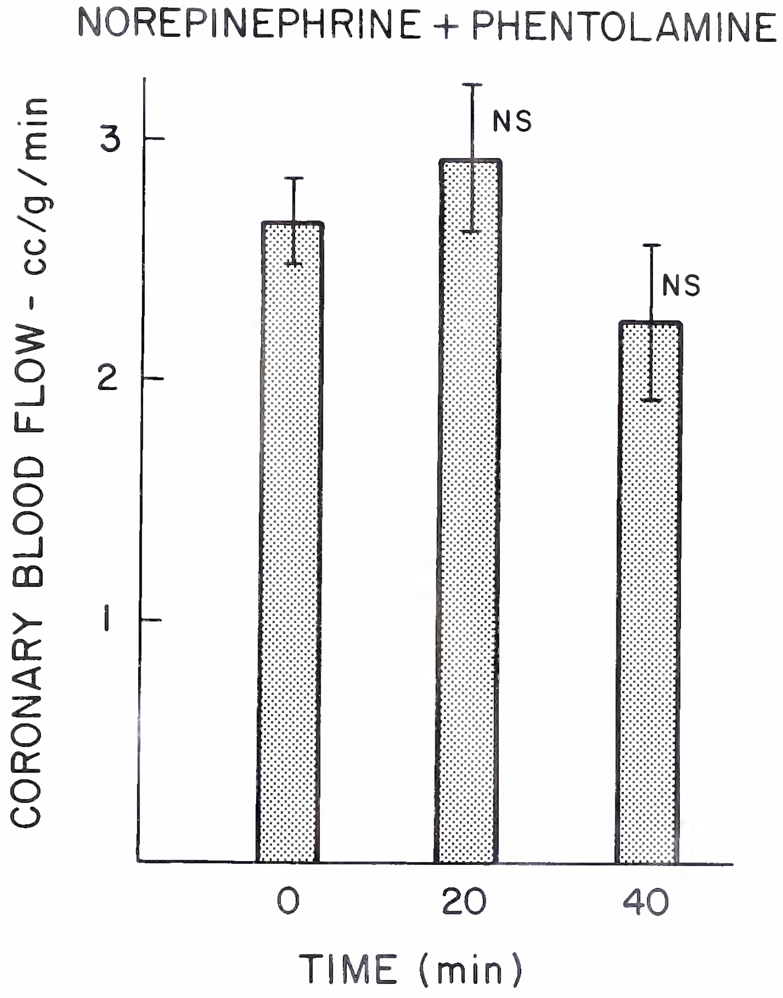
Coronary blood flow (CBF), cc/g/min, measured at four time intervals in a group of animals infused with NE. Vertical brackets, SEM.

FIGURE 4



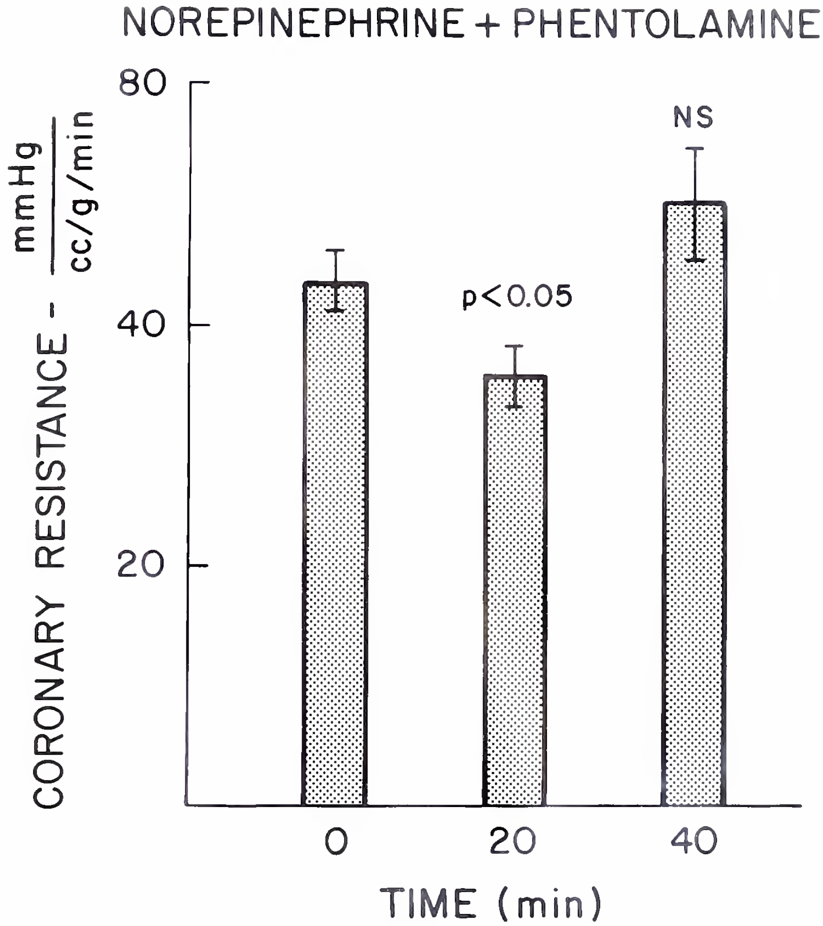
Coronary resistance, mm Hg/cc/g/min, in animals infused with NE is shown at four time intervals. Vertical brackets, SEM.

FIGURE 5



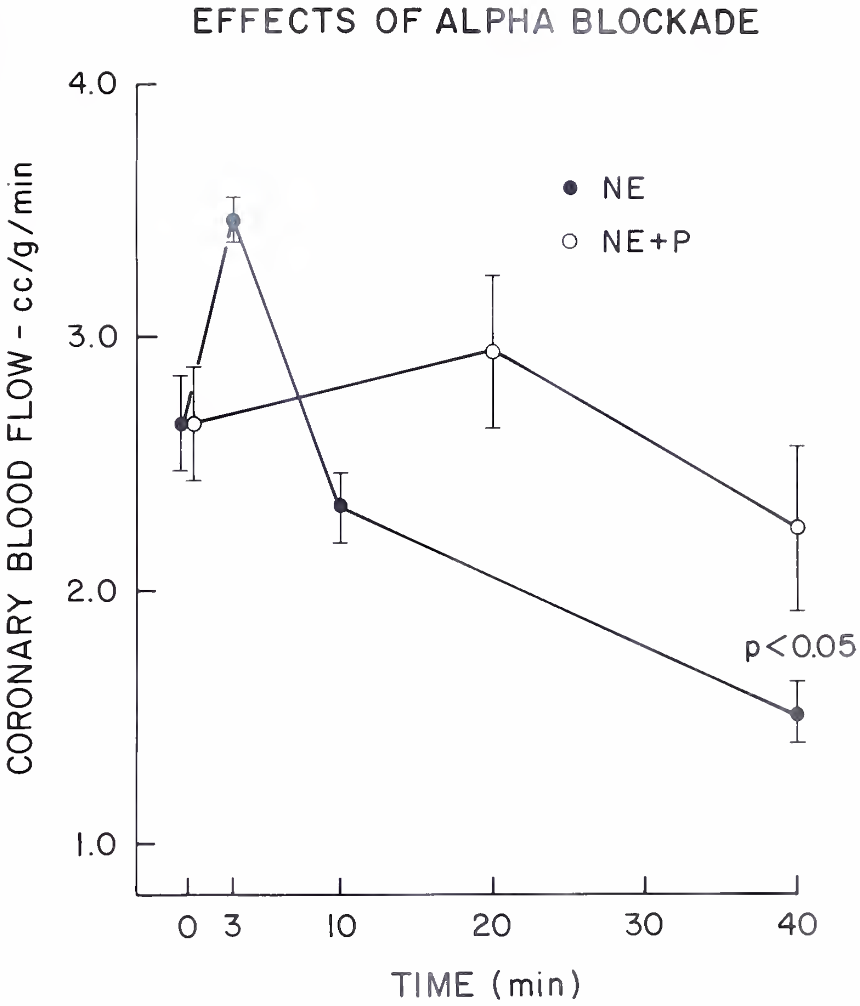
Coronary blood flow (CBF), cc/g/min, in animals pretreated with phentoalmine. Vertical brackets, SEM.

FIGURE 6



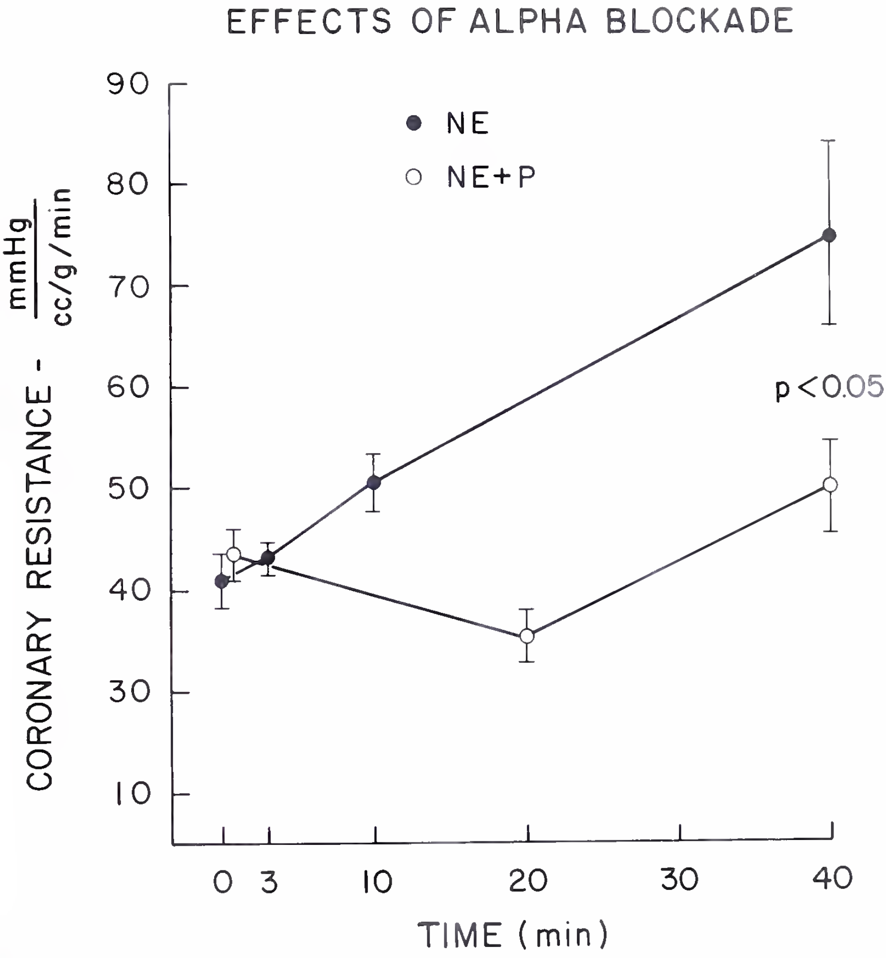
Coronary resistance (CR), mm Hg/cc/g/min, in animals pretreated with phentolamine. Vertical brackets, SEM.

FIGURE 7



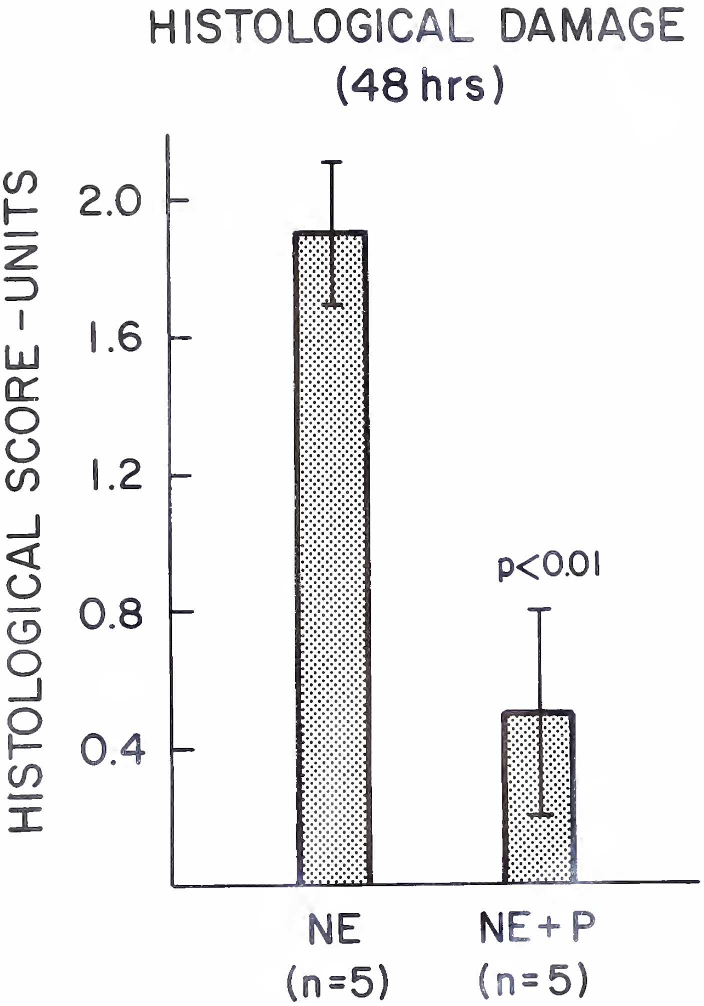
Coronary blood flow (CBF) at different time intervals in animals pretreated (open circles) and not pretreated (closed circles) with phentolamine. Vertical brackets, SEM.

FIGURE 8



Coronary resistance (CR) at different time intervals in phentolamine pretreated (open circles) and not pretreated (closed circles) animals. Vertical brackets, SEM.

FIGURE 9



Histogram of histological injury scores. NE-treated animals- left bar, NE+Ph treated animals- right bar. Vertical brackets, SEM.

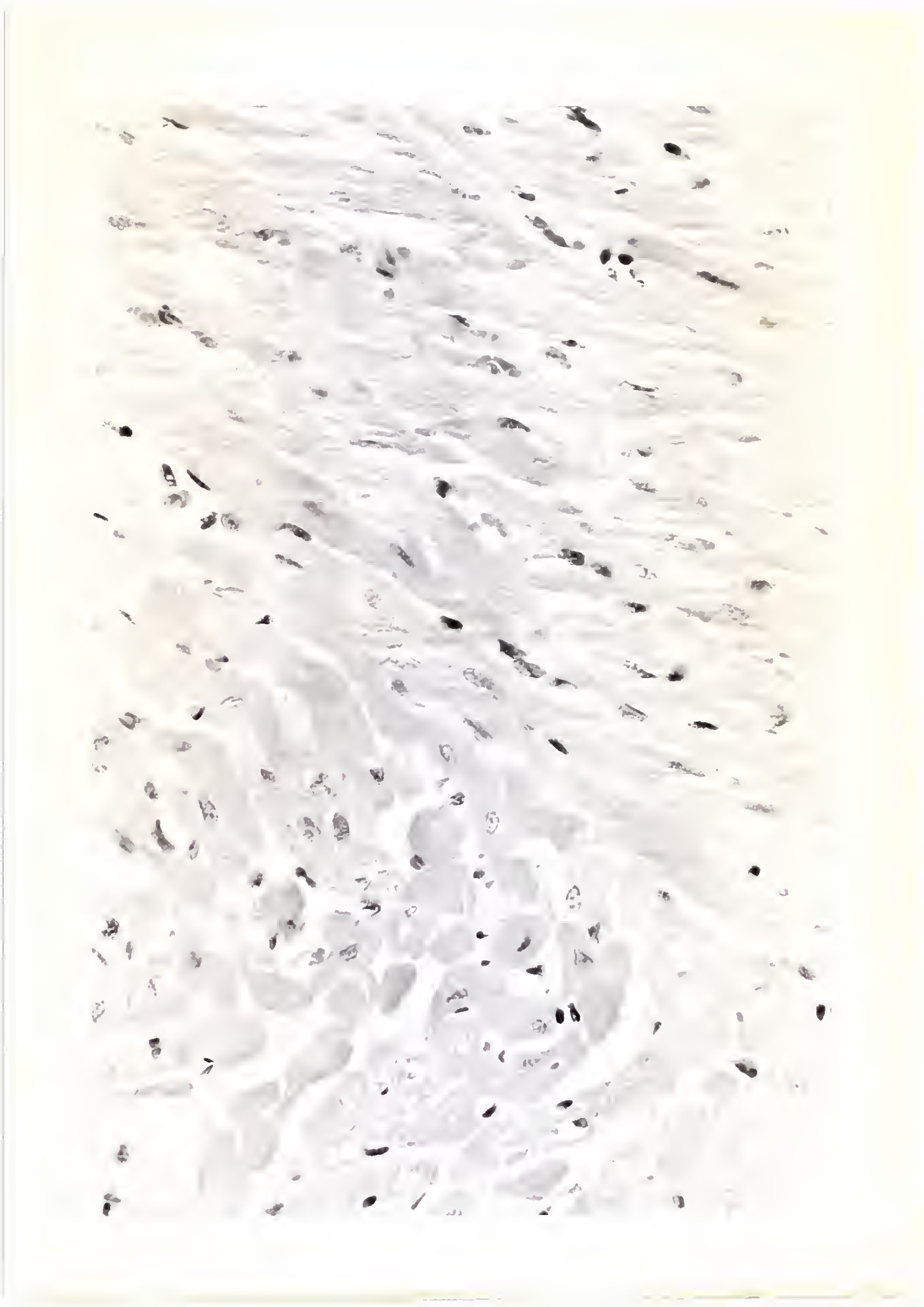
FIGURE 10

Microphotograph of myocardial section from a rabbit sacrificed 48 hrs after NE infusion (3 g/kg/min). H & E stain. Original magnification, x400.

FIGURE 11

Microphotograph of myocardial section from a rabbit pretreated with phentolamine and sacrificed 48 hrs after NE infusion (3 g/kg/min). H & E stain. Original magnification, x400.

FIGURE 12



Microphotograph of myocardial section from a rabbit pretreated with phentolamine and sacrificed 48 hrs after NE infusion (3 g/kg/min). Border zone between undamaged and damaged tissues. H & E stain. Original magnification, x400.

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